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SELECTING AND PROPAGATING CLONES OF BIGTOOTH MAPLE

(*ACER GRANDIDENTATUM* NUTT.)

by

Melody Reed Richards

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

Larry A. Rupp
Major Professor

V. Philip Rasmussen
Committee Member

Roger Kjelgren
Committee Member

Byron R. Burnham
Dean of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2010

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ABSTRACT

Selecting and Propagating Clones of Bigtooth

Maple (*Acer grandidentatum* Nutt.)

by

Melody Reed Richards, Master of Science

Utah State University, 2010

Major Professor: Dr. Larry A. Rupp
Department: Plants, Soils, and Climate

Numerous wild bigtooth maple (*Acer grandidentatum* Nutt.) specimens in northern Utah have potential for use in landscapes, but improvements in selection and propagation need to be developed before these specimens can be introduced to the green industry. Criteria-based evaluations centered on aesthetics, function, and fall color were performed to objectively select superior bigtooth maple specimens. Out of 56 trees initially selected for red fall color, six were selected for propagation based on all three criteria. Five of the six selected trees yielded viable bud take via chip budding. Optimum time for chip budding propagation was determined by four experiments. Coppiced seedling rootstocks were used with the “return budding” of excised buds as scions to parent stock (2006) and grafting buds from wild trees as scions (2007 and 2009). A fourth experiment examined chip budding of wild scions on 2-year-old, containerized, seedling rootstocks. The general time period identified as the optimum

time for budding bigtooth maple was July through mid-August. Propagation by cuttings was also explored as an alternative production method among bigtooth maple selections. Softwood cuttings were taken from six selections of wild bigtooth maples grafted on seedling rootstocks growing in a coppiced stool bed environment. Open-ended, black, velour, drawstring bags were placed over the end of pruned shoots at bud swell to initiate etiolation of the cuttings. The bags were left in place during shoot elongation to insure etiolation of the shoot base. Cuttings were harvested after 3 to 4 weeks, wounded, dipped in auxin, and placed on heating mats under an intermittent mist system. Rooting was evaluated on the cuttings after four weeks. Results showed the effects of etiolation to significantly increase the percentage of rooted cuttings and the number of roots per cutting.

(109 pages)

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Melody Reed Richards

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CHAPTER I

INTRODUCTION

Acer grandidentatum Nutt., commonly known as canyon or bigtooth maple, is native to the Intermountain West and has large populations in the foothills and mountains of northern Utah and southern Idaho (elevations ranging from 1300-2800 m). Bigtooth maple has often been confused as a subspecies of the eastern sugar maple (*Acer saccharum*), but has consistently been recognized in the western floras at the species rank (J. Wiersema, personal communication). Bigtooth maple possesses characteristics desirable for the landscape industry, such as drought tolerance, a wide range of soil and habitat tolerances, cold hardiness, and vibrant fall colors of yellows, oranges, and reds. Bigtooth maple also has opposite, simple, deeply-lobed, tooth-like leaves. The seeds vary in colors from green to red to brown. The genetic variability in bigtooth maple causes a range of naturally-occurring forms, such as short to tall, columnar to pyramidal to round, and multi-stemmed to single-trunk trees. The characteristics of this maple have sparked an interest in selecting superior clones and introducing them as a profitable addition to the nursery industry, as well as adding a pleasant western touch to local landscapes (Kuhns, 2003).

Nursery growers need to be aware of current trends and predict what trees will be commercially in demand for the future in their industry (Iles and Vold, 2003). We predict small, drought-tolerant trees will be in high demand within the nursery industry in the Intermountain West for water-conserving landscapes in the future. Bigtooth maple is rarely used for low-water, naturalized landscapes because of its lack of availability. Currently, two bigtooth maple cultivars have been selected and released to the nursery

industry. Rocky Mountain Glow[®] (*A. grandidentatum* ‘Schmidt’) is grown on sugar maple rootstock, produces yellow to orange-red fall color, and is commercially available (J. Frank Schmidt Catalog). Western Torch Wasatch Maple (*A. grandidentatum* ‘Western Torch’) is a newly released, multi-stemmed variety grafted onto the same species that produces red fall foliage but has limited availability (<http://greatwestintro.com/gwipage6.html>). Many wild specimens in northern Utah possess superior and unique characteristics, such as brilliant red fall color and columnar forms, and would be a great asset to the nursery industry. We expect more superior bigtooth maple specimens will help satisfy future demands of western landscape companies (Kuhns, 2003).

To fit the niches of the landscape industry, superior bigtooth maple specimens need to be identified and selected. Thousands of trees grow in such remote areas that it is impractical to evaluate each specimen on foot or by vehicle. Aerial photography or satellite photographs are a means of locating populations or individual trees. A logical way of selecting bigtooth maple is taking aerial digital images of diverse populations, identifying specimens by visually evaluating the image, and then finding the trees on the ground.

We hypothesize that a suitable means of introducing bigtooth maple clones to the nursery industry is to choose superior specimens in the wild based on aesthetic, function (Mee et al., 2003), and fall color traits that can be asexually propagated in a nursery environment. Bigtooth maple is presently propagated sexually by seed (Barker, 1975), but seedling propagation is unreliable for cloning purposes due to variable genetic

outcomes. Asexual propagation is ideal because it produces uniform clones that retain the superior traits of the parent plants desired by nurseries, landscapers, and homeowners (Henry and Preece, 1997). However, bigtooth maple is a hard-to-root species and asexual propagation can be challenging (Donnelly and Yawney, 1972; Tankersley, 1981).

Research on micropropagation of bigtooth maple is currently being conducted at New Mexico State University. Only greenhouse-grown seedling material can be used as propagules because field-grown or wild, mature trees cannot be used due to contamination issues (Bowen-O'Connor et al., 2006). Efforts in cutting propagation of bigtooth maple have also demonstrated low success (Tankersley, 1981). We predict that optimized budding of wild bigtooth maple scions is an effective option for establishment in the nursery. Further asexual experiments, such as cuttings, can be carried out once wild selections are growing in a nursery environment. Efficient asexual propagation will provide a greater number of improved clones for the nursery industry.

The first step in establishing selected trees is successfully grafting buds from wild trees onto a seedling rootstock in a nursery environment. Budding is a challenge in bigtooth maple due to factors such as small budwood, water-stressed buds, and the lack of budding skills, which all may contribute to low budding success rates. Unpredictable timing for greatest budding success in northern Utah is another factor. Budding success can be improved by optimizing factors, such as time of budding, to overcome challenges such as water stress or difficult morphology. Our objective was to find the optimum time for chip budding wild bigtooth maples in northern Utah in order to establish specimens from the wild.

Another method of asexual propagation is cuttings. However, research has shown that rooting bigtooth maple cuttings is difficult (Tankersley, 1981). We predict that propagation by cuttings has a greater chance for success if stock plants are in a controlled environment where growth can be optimized. Therefore, successful establishment of wild specimens in the nursery by budding is a prerequisite to further research to improve the propagation success of cuttings. We hypothesize that the rooting of bigtooth maple cuttings can be improved via the method of etiolation (light exclusion) based on the research of Maynard and Bassuk (1985, 1987). They demonstrated that rooting cuttings of hard-to-root species can be enhanced using the method of etiolation and auxin. The combination of etiolation and auxin increased rooting from 7% to 34% for *Acer griseum*, from 47% to 86% for *Acer saccharum*, from 0% to 100% for *Castanea mollissima*, from 51% to 100% for *Betula papyrifera*, and from 0% to 94% for *Carpinus betulus* (Maynard and Bassuk, 1985). Herman and Hess (1963) also indicated that the average number of roots was greater in etiolated, auxin-treated tissue than non-etiolated, auxin-treated tissue. The average number of roots per cutting for red kidney beans (*Phaseolus vulgaris*) was 1.4 for non-etiolated, auxin-treated cuttings and 125.4 for etiolated, auxin-treated cuttings (Herman and Hess, 1963). We expect that applying the combination of etiolation and auxin to bigtooth maple cuttings will cause a substantial improvement in rooting response and be a rewarding contribution to the nursery industry.

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CHAPTER II

SELECTION OF BIGTOOTH MAPLE

(*ACER GRANDIDENTATUM* NUTT.)

ABSTRACT

Numerous bigtooth maple (*Acer grandidentatum* Nutt.) specimens in northern Utah possess superior characteristics, such as brilliant red fall color and columnar forms that will be a great asset to the nursery and landscape industries. Evaluations based on aesthetics, function, and fall color were performed to objectively select superior bigtooth maple specimens. The aesthetic evaluation included taking aerial photographs using digital cameras (Nikon® D40 or D60) synced to a handheld GPS unit (Garmin™ GPSMAP 60C or GPSMAP 60CSx) while flying in a small airplane during peak fall color in Fall 2007 and 2008. The digital images and GPS coordinates were loaded onto GeoSetter® software to determine the latitude and longitude of the digital image based on GPS track files. Google™ Earth accurately identified the precise latitude and longitude of the bigtooth maple specimens in the digital image. The identified trees were located using GPS technology, and then visually assessed on the ground. Function evaluation criteria included appraising habitat, disease resistance, insect resistance, damage, bud quality, and the propensity toward a central leader or multiple stems from natural layering. The fall color evaluation documented the fall foliage color of the selected bigtooth maple specimen. Six out of 56 trees were selected for further propagation

testing based on the criteria from the three evaluations. Five of the six selected trees were successfully propagated via chip budding.

Introduction

Acer grandidentatum Nutt., commonly known as bigtooth maple possesses many characteristics desired by the landscape industry (Barker, 1974; Kuhns, 2003; Tankersley and Emino, 1981). The distribution spans ten western states with a large population throughout northern Utah and southern Idaho (elevations of 1300-2800 m). The characteristics of the bigtooth maple are that it is a small, deciduous tree (Eastmond, 1968) cold hardy (-35 to 38 °C) (Kuhns, 2003), drought tolerant (38 to 51 cm of annual precipitation), and adapts to a wide range of soils and habitats. A winged-samara fruit is produced in the summer. The leaves are simple, tooth-like, and deeply-lobed. The fall foliage is an assortment of reds. The genetic diversity among bigtooth maple causes many different canopy forms, such as columnar, pyramidal, and round. The above characteristics are ideal for selecting superior clones for the nursery industry.

The selection process not only involves considering characteristics of a tree, but considering the value of aesthetics and function the tree contributes to a landscape. The aesthetics and function of a landscape are essential requirements that have been integrated into public policy for landscape regulations (Chenoweth and Gobster, 1990). The interaction between aesthetics and function add a balance to the landscape (Tress et al., 2001). Bigtooth maple possesses various aesthetic and functional qualities that provide the needed balance for the landscape. For example, the simple, tooth-like leaves

are not only adapted to the arid climates of the Intermountain West, but the varying sizes of the dissected leaves add texture to the overall tree. Favorable aesthetic qualities among bigtooth maple such as leaves adding texture and form are key characteristics in selecting superior specimens for the landscape industry.

Bigtooth maple possesses a trait that is coveted by the landscape and nursery industries. Bigtooth maple produces brilliant, red-colored leaves in the fall. Color is one of the most important aesthetic qualities of a landscape. One of the top-selling trees in Iowa during 2003 was the red maple (*A. rubrum*) (Iles and Vold, 2003). Red is more preferred over yellow or orange (Guilford and Smith, 1959), and a study performed in 1941 showed that of all colors, red is the second highest preferred color, blue being the first, among men and women (Eysenck, 1941). In 2009, a study concluded that red was a preferred color among their participants when shown in a friendly, hospitable environment (Maier et al., 2009), such as a landscape. Many wild specimens in northern Utah produce red fall foliage that would be a great asset to the nursery and landscape industries. We expect that more superior bigtooth maple specimens will help satisfy the demands and future trends of western landscape contractors, nurseries, and consumers (Kuhns, 2003).

Function is a characteristic that, like aesthetics, is essential in selecting plants for the landscape. The functionality of bigtooth maple in a landscape includes habitat, disease, and insect tolerances. Bigtooth maple adapts well to a wide range of soils, is moderately shade- and cold-tolerant (USDA Plant Hardiness Zone 3), and is relatively free of serious disease and insect issues. Anthracnose (*Kabatella apocrypta*) and tar spot

(*Rhytisma*) are common diseases among bigtooth maple. Insects of concern are the twig borer (*Proteoteras aesculana*) (Solomon, 1995), aphids (*Aphis gossypii*), and cicadas (*Tibicen pruinosa*) (Rupp, 2008), but are considered to be a minor issue. Therefore, the minor disease and insect issues and adaptability to drought, cold, and a vast range of soils and habitats make bigtooth maple a functional tree for the landscape industry.

Proper selection of a native tree is difficult, but is made easier using aerial digital photography. Thousands of trees in remote areas make evaluating each specimen on foot or by vehicle challenging and impractical. Aerial photography or satellite images are a means of locating populations or individual trees and eliminating the necessity of evaluating each specimen on foot or by vehicle (Rupp, 2008). Natural color aerial photos provide the best means for mapping out forest habitat (Balice, 1979) and are significantly more correct than black and white (panchromatic film) aerial photographs (Heller et al., 1964). A 2001 study suggested that the best time to take aerial photos of deciduous trees is during peak fall color (Key et al., 2001). We submitted that a logical way of selecting bigtooth maple trees was taking aerial digital, real-color images of diverse populations from an airplane, visually evaluating the images, and then hiking to the identified tree on the ground.

We hypothesized that a suitable means of selecting and establishing bigtooth maple clones was to choose superior specimens in the wild based on aesthetics, function (resistance/tolerance criteria), and fall color, and then propagate them by budding. Asexual propagation is ideal because it produces uniform daughter plants that retain the superior traits of the mother plants desired by nurseries, landscapers, and homeowners

(Henry and Preece, 1997). Effective asexual propagation provides a greater number of improved clones for the landscape industry. To propagate large numbers of selected, wild trees we needed to successfully graft buds from those trees onto seedling rootstock in a nursery environment. Our goal was to have at least one successful bud take from each wild bigtooth specimen for potential use in the landscape industry.

Materials and Methods

Selection Process

Our objective in selecting wild bigtooth maple specimens was carried out using three evaluations: 1). Aesthetic/visual, 2). Function/characteristics, and 3). Fall color.

Aesthetic/Visual Evaluation

An aesthetic/visual evaluation was accomplished by taking aerial digital photos of diverse bigtooth maple populations in Cache Valley, UT, and then visually identifying unique bigtooth maple specimens from each image based on fall color and isolation. Trees were then physically located on the ground and the tree form was evaluated.

Three different cameras were used to take aerial photographs of bigtooth maples during peak color in Fall 2007 (Fig. 1):

- 1) Free hand images were taken from a Piper Cub on 11 Sept. 2007 with a Fujifilm S3 Pro UVIR camera on RAW level with an image-stabilized "HAD-CCD" (hole accumulation diode, charge coupled device) sensor digital color camera.



Fig. 1. Three types of aerial images from Fall 2007. A). Aerial image taken with a Fujifilm S3 Pro UVIR camera on RAW level with an image-stabilized "HAD-CCD." B). Free hand real-color digital images taken with a Nikon® D40 camera. C). Aerial image taken with a Kodak Megaplex 4.2i Digital Frame Cameras (2 K x 2 K pixel sensor) with interference filters forming bands in the green, red and blue bands.

- 2) Free hand real-color digital images were also taken on 11 Sept. 2007 with a Nikon[®] D40 camera.
 - 3) Fixed camera aerial photographs of predetermined transects (based on Google[™] Earth images) were taken on 11 Oct. 2007 with three Kodak Megaplug 4.2i Digital Frame Cameras (2 K x 2 K pixel sensor) with interference filters forming bands in the green, red, and blue bands to obtain RGB real color composites. The camera was equipped with a GPS locator. Images were recorded on a Pentium[®] computer with a 40 GB drive, 20 GB removable drives, a DVD/CD ROM backup system, and EPIX[™] controller boards using in-house developed software. Images were reviewed visually using ERDAS Imagine[®] 9.1 to identify trees portraying exceptional fall color. Trees were identified with a 255 spectral value (highest red color content in pixel). The identified trees in the aerial images were corrected for geometric and radial distortions, lens vignetting, and radiance non-uniformities. Geo-registering (rectifying) to digital orthophoto quadrangle base maps was used to determine the exact latitude and longitude of images.
- Only one method of aerial photography was used to capture images of bigtooth maples during peak color in Fall 2008:
- 1) Real color images were taken from a Piper Cub on 30 Sept. 2008 and 2 Oct. 2008 with a Nikon[®] D60 or D40 camera (18 to 55 mm lens). Approximately 200 pictures were taken on each flight. Flight transects included the Wellsville and Bear River Mountain Ranges in Cache Valley, UT.

- 2) Time metadata from images taken with Nikon[®] D40 and D60 cameras was synchronized with the time on either a Garmin[™] GPSMAP 60C or GPSMAP 60CSx with GeoSetter[©] software to record the geographic coordinates of the airplane at the time each digital aerial image was taken.
- 3) Trees were identified for further evaluation based on color and form. The exact latitude and longitude of the identified trees were found using Google[™] Earth in conjunction with the coordinates of the aerial photographs as provided by GeoSetter[©].

In Summer 2008 and 2009, Google[™] Earth images of the identified trees from aerial photographs were printed and the coordinates entered into a Garmin[™] GPSMAP 60CSx. Additional trees were also located via personal communication with Jerry Morris. Hikes to the identified trees were carried out during optimal budding time from early July to mid-August 2008 and 2009 (Chapter 3). A vehicle was driven to the general location of the tree. We then walked cross-country for distances ranging from five meters to five kilometers. The specimen was visually evaluated for its potential as a landscape specimen.

Tree form was the last part of the aesthetics/visual evaluation. Round and pyramidal forms were considered aesthetically acceptable. Those with non-uniform branching were considered aesthetically unacceptable. Unique forms, such as columnar, automatically were kept for further evaluation.

Function/Characteristics Evaluation

A second evaluation based on function and characteristics was performed if the tree met the criteria for the initial aesthetic/visual evaluation. The first part of the evaluation was to take photographs of the bigtooth maple specimen. All angles of the tree (north, east, south, and west) were documented using a Canon[®] Powershot SD750 Digital ELPH camera to document the overall tree form. Images of the leaves, trunk(s), buds, and fruit were also recorded. The second part of the evaluation consisted of a list of criteria for function and characteristics of the tree, enabling the second evaluation to be more consistent and less subjective (Dana, 2000). The following criteria used were from the USDA-ARS Germplasm Resources Information Network (GRIN) (www.ars-grin.gov/sitemapgrin.html):

1. Habitat
2. Foliar Disease (Anthracnose)
3. Insect damage (Twig borer, cicadas, aphids)
4. Quality of budwood
5. Layering
6. Leaf length
7. Leaf width
8. Tree height
9. Tree crown width
10. Trunk circumference
11. Fruit length

12. Fruit width

The features considered for the function evaluation from the list of criteria were habitat, disease, insect damage, quality of budwood, and layering.

- 1) Native habitat is an important indicator of the potential success of clones of selected plants in a landscape. Native maples exist in a wide range of upland habitats ranging from riparian areas to dry hillsides.
- 2) Anthracnose and tar spot are common diseases present on the leaves of bigtooth maples.
- 3) Insects of concern were cicadas, aphids, and twig borers. Cicadas commonly oviposited in small shoots resulting in the death of some shoots and reduced quality of buds for propagation. Cicadas generally did not damage all the budwood on the tree. Aphids appeared in the fall and left a sticky exudate on the leaves. Twig borers were rarely present among the bigtooth maple. Small holes were detected in the buds when twig borers were present.
- 4) The quality of the budwood (terminal shoots of current budwood of season with one or more sets of lateral buds and a terminal bud) was essential for cloning the selected bigtooth maple. A visual evaluation was carried out to determine the bud quality. If the budwood contained at least two vigorous buds, then the budwood was considered high quality.
- 5) The appearance of multiple trunks is found among bigtooth maple due to natural layering of lower branches. Layering is advantageous in that it produces a native look and disadvantageous in that it increases the need for maintenance. A more

central leader with fewer layers requires less maintenance and is considered more functional in a landscape.

Characteristics from the list of criteria included leaf length, leaf width, tree height, tree crown width, trunk circumference, fruit length, and fruit width. An average-sized leaf was chosen and length and width were measured using a ruler. The tree height was measured using a meter stick by standing far away from the tree so as to view the tree without having to move your head to see the whole tree. The meter stick was then stretched forth in front at arm length (hold meter stick perpendicular to ground, holding at the number of centimeters at arm length), with one eye closed, and then lined up with the tree by walking back. The tree height was then measured by measuring how far one had to walk backwards in order to line up the meter stick. The tree crown width was measured with a measuring tape on the ground, recording the east/west measurement and the north/south measurements. The trunk circumference was measured at chest height (1.2 m) on all trunks of all trees in order to keep consistency. The fruit length and width were measured using a ruler on an average-sized samara. If all the measurements and functionalities of the bigtooth maple specimen were considered acceptable, then the third evaluation was carried out.

Fall Color Evaluation

Hikes to the selected bigtooth maple trees were carried out in late September and early October. Fall leaf color was evaluated by taking pictures with a Canon[®] Powershot SD750 Digital ELPH camera. A Munsell color chart was included in each picture to accurately document the color of the trees (Appendix A). Leaf samples were also

collected and pressed for color comparison (Appendix A). The bigtooth maple trees with the most brilliant reds were considered ideal and selected for cloning in the nursery environment.

Cloning in Nursery Environment

Budwood from the wild bigtooth maple specimens provided buds for chip budding scions onto bigtooth maple seedling rootstocks at the North Logan, UT Greenville Farm. Budding was done in July and August of 2008 and 2009. Nine to seventeen budsticks of current season's wood were collected from the main canopy of each tree at chest height. The leaves and part of the petiole were immediately cut off the budstick. Budsticks were placed in plastic, quart-sized bags in a cooler of ice for storage until grafting was performed. Chip budding was performed by excising a 2 cm long chip from the budwood and inserting it into a 2 cm slot previously cut on the seedling rootstock in which the rootstock stem diameter was equal to or slightly larger than the budwood. Only one bud per budstick node was used. The petiole was cut off just above the bud and then left affixed to the bud shield, resulting in better protection of the bud. Parafilm[®] Grafting Tape was used to hold the buds in place and prevent desiccation. Buds from each tree from the wild were budded onto one rootstock tree at the Greenville Farm. Budding was done within 24 h of excising the budwood from the selected, wild tree, but usually within 2 to 3 h of collection. After three weeks, the Parafilm[®] Grafting Tape was slit to prevent potential girdling of the shoot by the tape, thus allowing the trees to grow the rest of the season. Final assessments of bud take were made in Spring 2009 and 2010 based on actual budbreak.

Results

Selection Process

The different methods of the 2007 aerial photography results are as follows:

- 1) The Fujifilm S3 Pro UVIR camera on RAW level with “HAD-CCD” sensors resulted in false color images that lacked the color variation needed to identify an extreme range of red trees.
- 2) The Kodak Megaplug 4.2i Digital Frame Camera system did not provide real-color images and needed to be manipulated to identify red portions of the spectrum. The images portrayed the trees at an extreme distance making identification of isolated trees difficult. The ERDAS Imagine[®] 9.1 program was tedious and time consuming. Georectification had to take place to obtain the coordinates of a tree in the selected image.
- 3) Standard digital camera images (Nikon[®] D40) also needed to be georectified to locate identified trees from the image because no GPS device was synchronized to the camera. However, the standard digital camera produced the best real color images.

A total of 30 trees were identified in Fall 2007 aerial photographs. None of Fall 2007 identified trees met the criteria of the three evaluations in Summer 2008. However, two trees (USU-ACGR-1022 and USU-ACGR-1032) were selected with the aid of Fall 2007 aerial photographs via field observation. The two selected bigtooth maple trees were found in close proximity to the identified tree in the aerial photograph. A third tree

(USU-ACGR-1034) was also selected in Summer 2008 through personal communication with Jerry Morris, a plantsman from Denver, Colorado.

In Fall 2008, we took free hand aerial photos of bigtooth maple trees with Nikon® D40 and D60 cameras and used GeoSetter© to locate the identified trees. The Fall 2008 method was more effective and practical compared to Fall 2007 methods of using various cameras and georectification to identify specimens. A total of 15 bigtooth maple trees were identified via Fall 2008 aerial photographs. Three of the 15 identified trees (USU-ACGR-1036, 1038, AND 1041) were selected in Summer 2009. The six selected trees from Summer 2008 and 2009 are as follows.

USU-ACGR-1022
 N 41.50242, W -111.95759
 Elevation: 1700 m
 (Fig. 2)

Aesthetic Evaluation

- 1). Aerial photo: N/A- Found en route to an identified, aerial-photographed tree, but not a tree identified by an aerial photograph.
- 2). Canopy form: Round

Function Evaluation

- 1). Habitat: Dry, south-facing slope. Non-riparian.
- 2). Disease: Anthracnose (minor).
- 3). Insects: Aphids (appeared in fall).
- 4). Bud quality: At least two vigorous buds on all collected budwood.
- 5). Layers: 5

Characteristics

- 1). Leaf length: 63.5-89 mm
- 2). Leaf width: 76-102 mm
- 3). Height: 5.5 m
- 4). Crown width: 5.18 x 5.18 m
- 5). Circumference: 2.26 m (46, 53, 38, 43, 46 cm)
- 6). Fruit length: 38 mm
- 7). Fruit width: 64 mm

Fall Color Evaluation

- 1). Color: Brilliant red covering the entire tree.
- 2). Peak color: 30 Sept. 2008
- 3). Leaf samples were collected and pressed (Appendix A).
- 4). Munsell color chart fall color comparison (Appendix A).

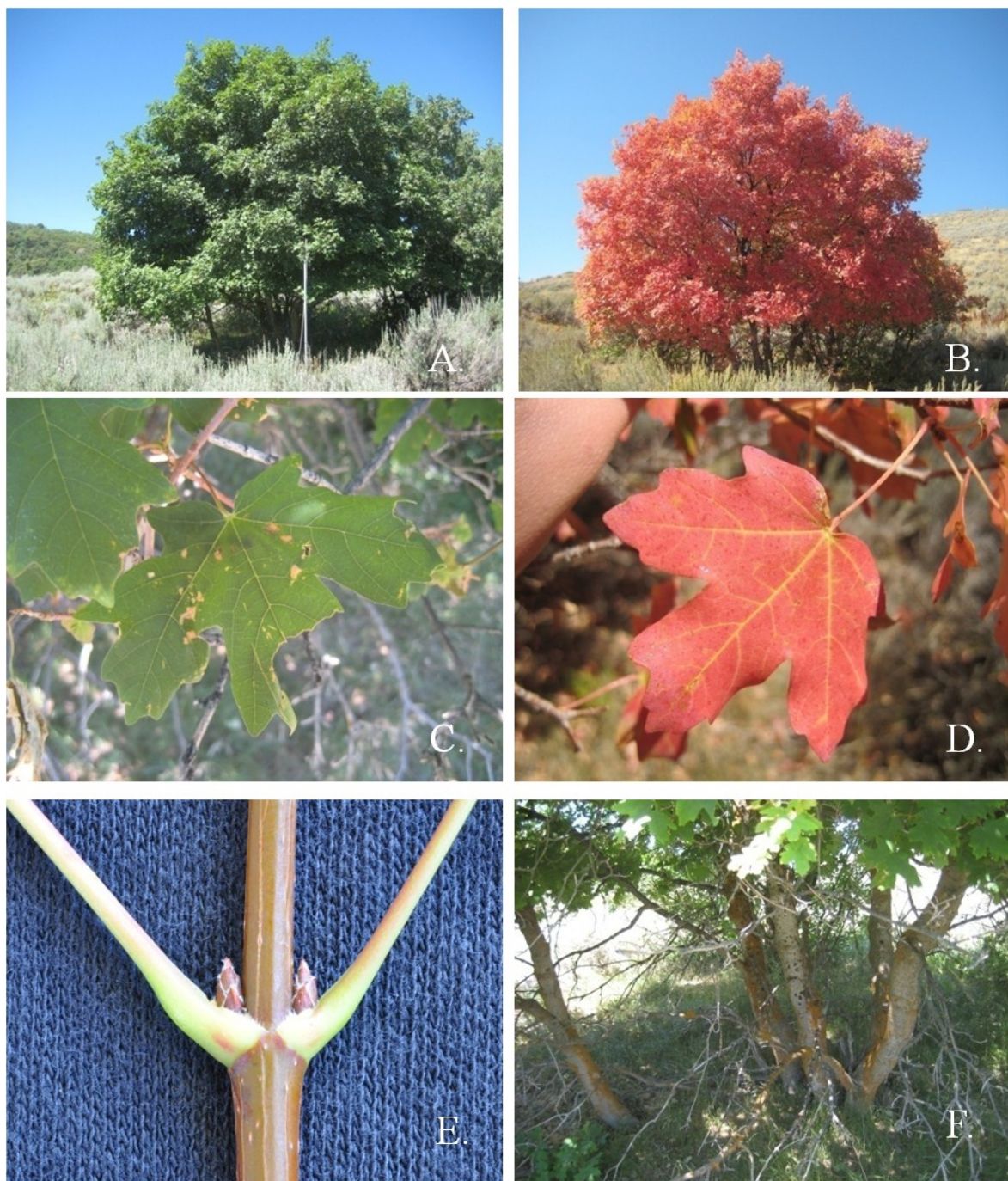


Fig. 2. Images of bigtooth maple USU-ACGR-1022. A). USU-ACGR-1022 in the summer. B). USU-ACGR-1022 in the fall at peak color. C). Leaf damage on USU-ACGR-1022 D). Red leaf in fall on USU-ACGR-1022. E). Budwood from USU-ACGR-1022 F). Five trunks of USU-ACGR-1022.

USU-ACGR-1032
 N 41.55753, W -111.90730
 Elevation: 1680 m
 (Fig. 3)

Aesthetic/Visual Evaluation

- 1). Aerial photo: N/A. Found en route to an identified, aerial-photographed tree.
- 2). Tree form: Columnar

Function Evaluation

- 1). Habitat: Dry, south-facing slope. Non-riparian.
- 2). Disease: Anthracnose (minor).
- 3). Insects: Cicadas (minor). Aphids (moderate).
- 4). Bud quality: Few on the tree, but at least two vigorous buds per budstick.
- 5). Layers: 6

Characteristics

- 1). Leaf length: 76 mm
- 2). Leaf width: 102 mm
- 3). Height: 10.7 m
- 4). Crown width: 3.7 x 3.35 m
- 5). Circumference: 2.54 m (46, 25, 56, 36, 53, and 38 cm)
- 6). Fruit length: 25 mm
- 7). Fruit width: 50 mm

Fall Color Evaluation

- 1). Color: Mainly green with patches of red.
- 2). Peak color: 26 Sept. 2008
- 3). Leaf samples were collected and pressed (Appendix A).
- 4). Munsell color chart fall color comparison (Appendix A).



Fig. 3. Images of bigtooth maple USU-ACGR-1032. A). USU-ACGR-1032 in summer. B). USU-ACGR-1032 in fall. C). Leaves with aphid honeydew in summer on USU-ACGR-1032. D). Fall leaves on USU-ACGR-1032. E). Budwood of USU-ACGR-1032. F). Six trunks of USU-ACGR-1032.

USU-ACGR-1034
 N 41.56179, W -111.94002
 Elevation: 1707 m
 (Fig. 4)

Aesthetic/Visual Evaluation

- 1). Aerial photo: N/A. Found by personal communication with Jerry Morris.
- 2). Tree form: Single trunk with round canopy.

Function Evaluation

- 1). Habitat: Dense grove of trees on north-facing slope.
- 2). Disease: Anthracnose (minor).
- 3). Insects: Twig borers (minor).
- 4). Bud quality: Very small, but at least two vigorous buds on each budstick.
- 5). Layers: 0

Characteristics

- 1). Leaf length: 102 mm
- 2). Leaf width: 127 mm
- 3). Height: 17.7 m
- 4). Crown width: 7.9 x 7.9 m
- 5). Circumference: 1.1 m
- 6). Fruit length: N/A
- 7). Fruit width: N/A

Fall Color Evaluation

- 1). Color: Mainly green with patches of yellows, oranges, and reds.
- 2). Peak color: 26 Sept. 2008
- 3). Leaf samples were collected and pressed (Appendix A).
- 4). Munsell color chart fall color comparison (Appendix A).



Fig. 4. Images of bigtooth maple USU-ACGR-1034. A). USU-ACGR-1034 in summer. B). USU-ACGR-1034 in fall. C). Leaves of USU-ACGR-1034. D). Fall leaves of USU-ACGR-1034. E). Budwood from USU-ACGR-1034. F). Single trunk of USU-ACGR-1034.

USU-ACGR-1036
 N 41.55967, W -111.90307
 Elevation: 1670 m
 (Fig. 5 and 6)

Aesthetic/Visual Evaluation

- 1). Aerial photo: Identified in aerial photo taken on 30 Sept. 2008 (Fig. 5). Chosen for brilliant red fall color and isolated from other trees.
- 2). Tree form: Oval

Function Evaluation

- 1). Habitat: Dry, south-facing slope, non-riparian.
- 2). Disease: Anthracnose (minor).
- 3). Insects: Cicadas (minor).
- 4). Bud quality: At least two vigorous buds per budstick.
- 5). Layers: 6

Characteristics

- 1). Leaf length: 64 mm
- 2). Leaf width: 75 mm
- 3). Height: 6.7 m
- 4). Crown width: 4.3 x 4.6 m
- 5). Circumference: 2.66 m (41, 58, 25, 64, 53, and 25 cm)
- 6). Fruit length: 38 mm
- 7). Fruit width: 38 mm

Fall Color Evaluation

- 1). Color: Brilliant red covering the entire tree.
- 2). Peak color: 29 Sept. 2009
- 3). Leaf samples were collected and pressed (Appendix A).
- 4). Munsell color chart fall color comparison (Appendix A).



Fig. 5. Aerial image of bigtooth maple USU-ACGR-1036.



Fig. 6. Image of bigtooth maple USU-ACGR-1036. A). USU-ACGR-1036c2 in summer. B). USU-ACGR-1036 in fall. C). Green leaves in summer of USU-ACGR-1036. D). Red leaves in fall of USU-ACGR-1036. E). Budwood from USU-ACGR-1036. F). Trunks of USU-ACGR-1036.

USU-ACGR-1038
 N 41.548280, W -111.908070
 Elevation: 1700 m
 (Fig. 7 and 8)

Aesthetic/Visual Evaluation

- 1). Aerial photo: Identified in aerial photo taken on 30 Sept. 2008 (Fig. 7).
 Chosen for brilliant red fall color.
- 2). Tree form: Oval

Function Evaluation

- 1). Habitat: Dry, south-facing slope.
- 2). Disease: Anthracnose (minor).
- 3). Insects: Cicadas (minor).
- 4). Bud quality: Very vigorous budwood with at least two vigorous buds per budstick.
- 5). Layers: 18

Characteristics

- 1). Leaf length: 75 mm
- 2). Leaf width: 75 mm
- 3). Height: 8.5 m
- 4). Crown width: 5.5 x 5.5 m
- 5). Circumference: 4.86 m (15, 25, 38, 20, 53, 30, 20, 36, 33, 43, 41, 15, 20, 28, 23, 20, 13, 13 cm).
- 6). Fruit length: N/A
- 7). Fruit width: N/A

Fall Color Evaluation

- 1). Color: Brilliant red
- 2). Peak color: Before 29 Sept. 2009
- 3). Leaf samples were collected and pressed (Appendix A).
- 4). Munsell color chart fall color comparison (Appendix A).



Fig. 7. Aerial image of bigtooth maple USU-ACGR-1038.



Fig. 8. Image of bigtooth maple USU-ACGR-1038. A). USU-ACGR-1038 in summer. B). USU-ACGR-1038 in fall. C). Green leaves in summer of USU-ACGR-1038. D). Red leaves in fall of USU-ACGR-1038. E). Budwood from USU-ACGR-1038. F). Trunks of USU-ACGR-1038.

USU-ACGR-1041
 N 41.739058, W -112.035877
 Elevation: 1610 m
 (Fig. 9 and 10)

Aesthetic/Visual Evaluation

- 1). Aerial photo: Identified in aerial photo taken on 30 Sept. 2008 (Fig. 9). Chosen for brilliant red fall color, tree form, and isolation.
- 2). Tree form: Pyramidal

Function Evaluation

- 1). Habitat: Above riparian area on dry, north-facing slope.
- 2). Disease: Anthracnose (minor).
- 3). Insects: Cicadas (minor).
- 4). Bud quality: Cicada damage, but enough budsticks with at least two vigorous buds.
- 5). Layers: 3

Characteristics

- 1). Leaf length: 100 mm
- 2). Leaf width: 125 mm
- 3). Height: 9.1 m
- 4). Crown width: 9 x 6.7 m
- 5). Circumference: 2.2 m (71, 78, 71 cm).
- 6). Fruit length: 38 mm
- 7). Fruit width: 50 mm

Fall Color Evaluation

- 1). Color: Brilliant red leaves covering the entire tree.
- 2). Peak color: 28 Sept. 2009
- 3). Leaf samples were collected and pressed (Appendix A).
- 4). Munsell color chart fall color comparison (Appendix A).

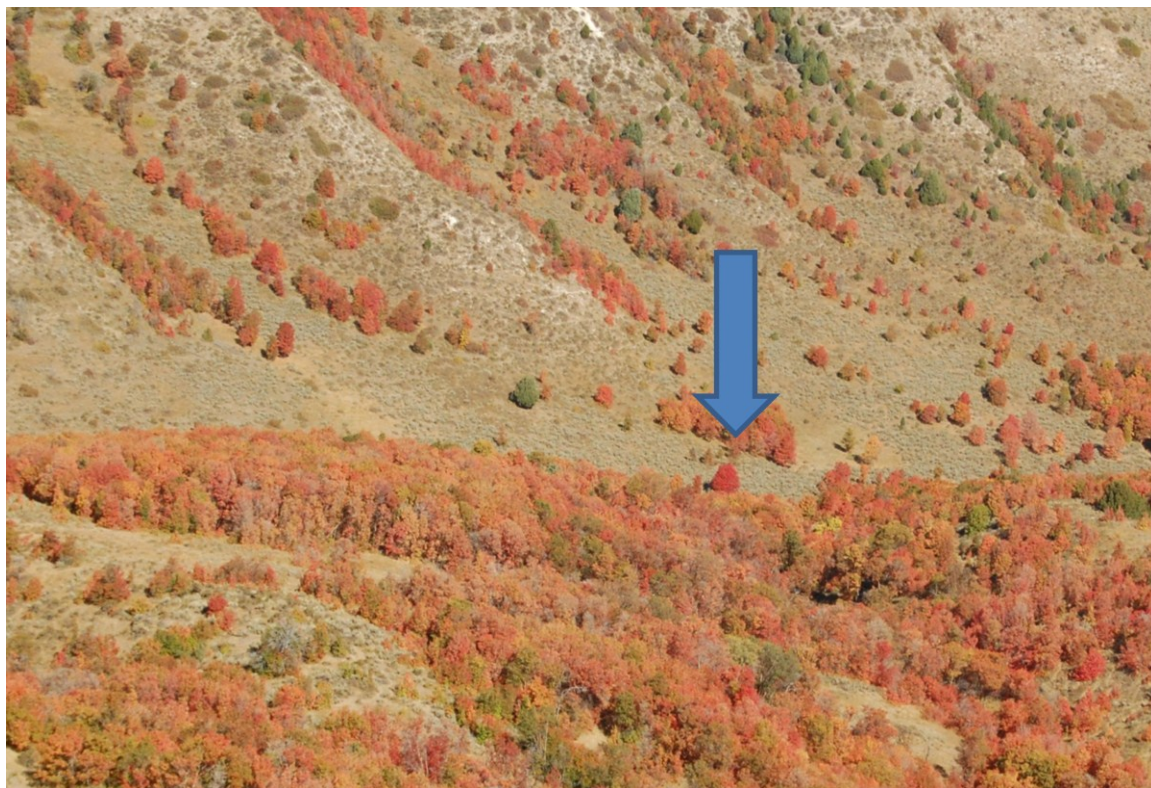


Fig. 9. Aerial image of bigtooth maple USU-ACGR-1041.



Fig. 10. Image of bigtooth maple USU-ACGR-1041. A). USU-ACGR-1041 in summer. B). USU-ACGR-1041 in fall. C). Green leaves of USU-ACGR-1041 D). Red leaves in fall of USU-ACGR-1041. E). Budwood from USU-ACGR-1041. F). Trunks of USU-ACGR-1041.

Cloning in Nursery Environment

Budwood was collected from all six bigtooth maple specimens and final assessments of bud take were made in Spring 2009 and 2010 based on actual budbreak. All of the trees had at least one successful bud take, except USU-ACGR-1041 (Table 1). The highest bud take percentage was from USU-ACGR-1022 with 56%. More buds from USU-ACGR-1041 were collected in Summer 2010 and the bud take will be evaluated in Spring 2011.

Table 1. Budding success rate for wild scion wood budded on nursery-grown, coppiced rootstocks.

Tree #	# of buds	Alive	Dead	%Success	Date budded
USU-ACGR-1022	9	5	4	56%	10-Jul-08
USU-ACGR-1032	17	9	8	53%	8-Aug-08
USU-ACGR-1034	15	4	11	27%	11-Aug-08
USU-ACGR-1036	10	4	6	40%	16-Jul-09
USU-ACGR-1038	12	1	11	8%	22-Jul-09
USU-ACGR-1041	12	0	12	0%	28-Jul-09
TOTAL	75	23	52	31%	

Discussion and Conclusions

The aesthetic, function, and fall color evaluations were valuable in selecting superior bigtooth maple specimens for clonal propagation. The most effective method in locating superior bigtooth maple trees was using free hand digital images taken from an airplane, synced with a GPS unit, and located using GeoSetter[®] and Google[™] Earth. Other methods, such as personal communication and finding superior trees in close

proximity to an aerial-photographed tree, were shown to be advantageous in the selection process as well. Assessing the identified tree in the aerial photograph and on the ground with the naked eye was practical in selecting trees that fulfill landscaping requirements. The list of criteria in the second evaluation appraised the function of the tree. Assessment of habitat, disease, insect, bud quality, and layering gave us a better indication of the qualifications of each tree for the landscape industry. Fall color was an essential evaluation because it is a clonable characteristic and is one of the most important qualities of a landscape tree. Cloning bigtooth maple via budding was a means whereby we could establish these wild specimens in a nursery environment. Overall bud take percentages were not high. However, five out of the six selected trees were successfully grafted. Establishment of these trees in the nursery will permit further research on their propagation, production, and landscape use.

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CHAPTER III

THE EFFECT OF BUDDING DATE ON SUCCESSFUL BUDDING OF BIGTOOTH
MAPLE (*ACER GRANDIDENTATUM* NUTT.)

ABSTRACT

Introduction of wild specimens of bigtooth maple (*Acer grandidentatum* Nutt.) into the nursery industry is dependent upon successful propagation. Successful first stage propagation requires facilitating improved budding procedures using wild scions on seedling nursery rootstock. We determined the optimum time for chip budding by conducting three time-course experiments using multi-stemmed, bigtooth maple seedling rootstocks in a coppiced stoolbed and one experiment using 2-year-old containerized bigtooth maple seedlings. A “return budding” experiment was completed in Summer 2006 in which buds were removed from coppiced bigtooth maple seedling shoots and then replaced into the precise location from which they were removed. The objective was to determine the optimum time for budding while minimizing the effects of budding technique. The results showed late June through July to have the highest bud take percentages. Buds from wild bigtooth maple specimens were used as scions on coppiced bigtooth maple shoots in Summer 2007 and 2009. Budding in early July through mid-August resulted in the highest bud take percentages in Summer 2007 and 2009. The last experiment was conducted in Summer 2009 using wild buds on 2-year-old containerized bigtooth maple seedling rootstocks. The results for Summer 2009 showed the highest bud take in July through mid-August. The combination of results indicates that July

through mid-August is the optimum time for chip budding bigtooth maple in northern Utah.

Introduction

Bigtooth, or canyon maple, (*Acer grandidentatum* Nutt.) is a small tree native to the Intermountain West and possesses desirable characteristics for the nursery industry (Barker, 1974; Kuhns, 2003; Tankersley and Emimo, 1981). Bigtooth maple is cold hardy (-35 to -38 °C) (Kuhns, 2003), drought tolerant (38 to 51 cm of annual precipitation), and adapts well to a wide range of soils and habitats. The leaves are simple, tooth-like, and deeply-lobed. Leaf colors such as reds, oranges, and yellows emerge in the fall (Barker, 1974, 1977). Genetic diversity among bigtooth maple causes different canopy forms such as columnar, pyramidal, and round. These characteristics and genetic diversity among bigtooth maple can be retained via asexual propagation.

Superior specimens of bigtooth maple with unique characteristics will satisfy the high demands and future trends of the nursery industry. Currently, two bigtooth maple cultivars have been released to the nursery industry. Rocky Mountain Glow® (*A. grandidentatum* ‘Schmidt’) is grown on sugar maple rootstock, produces yellow to orange-red fall color, and is commercially available (J. Frank Schmidt Catalog). Western Torch Wasatch Maple (*A. grandidentatum* ‘Western Torch’) is a newly released, multi-stemmed variety grafted onto the same species, produces red fall foliage, and has limited availability (<http://greatwestintro.com/gwipage6.html>). Many wild specimens in northern Utah also possess superior and unique characteristics that can be propagated, such as brilliant red fall color and columnar forms, and would be a great asset to the

nursery industry. We expect that propagating superior bigtooth maple specimens via budding will initiate further research of asexual propagation with bigtooth maples for nursery production.

Budding is a logical process for cloning bigtooth maple trees from the wild into a nursery environment, but there is need for improving the budding process in maples. Low percentages of successful unions are common among maples (Howard, 1973). The diameter of the budwood on native, Utah maples is relatively small making removal of the bud difficult. A lack of budding skills can also result in low proportions of successful unions (Alsup et al., 2003). However, the budding process can be improved. Chip budding has been shown to have a three-fold increase in bud take compared to T-budding (Howard, 1973) and is one of the best budding methods for woody species; as it is simple and quick to perform (Hartmann et al., 2002). Budding at the right time of year has also shown improvement in bud take among maples (Howard, 1973). Our objective was to find the optimum time for the highest bud take of wild bigtooth maples via chip budding.

Materials and Methods

A block of coppiced, multiple-stemmed seedling maples was established in 1999 and used as rootstocks for most of the budding experiments (Fig. 11). The rootstocks were used as an initial repository for budwood collected and grafted (chip bud graft) from various wild specimens. The seedlings were pruned back to the crown every year (starting in 2001) to induce vigorous shoot growth. Ammonium sulfate 21N-0P-0K fertilizer (2.44 g m^{-2}) was applied to the coppiced bed annually (starting in 2004).

Irrigation was applied twice daily for 10 min from July to mid-September. A layer of conifer wood shavings also covered the ground around the rootstocks.



Fig. 11. Images of coppiced bigtooth maple seedlings used as rootstocks for budding experiments.

Experiment 1: The Effect of Budding Date on “Return Budding” Success in 2006-07

The optimal time of successful bud take among bigtooth maple in northern Utah was tested in the Summer 2006. A “return budding” experiment was conducted in which buds were removed from shoots of coppiced seedlings, and then budded back into the precise location from which they were removed. The objective was to determine optimum budding time while decreasing the effects of budding technique on bud take. Fifteen coppiced plants were selected based on the presence of multiple shoots of moderately vigorous growth. No random assignment of shoots was conducted previously to the budding dates due to the inability to predict later season growth patterns. The most ideal shoot was selected for budding on each specified budding date using dormant buds from the second node above the base. An approximately 2 cm long chip was removed by

making a 45° angle cut into one-third of the diameter of the stem, and then making another incision proceeding linearly from a point approximately 2 cm above the first cut to the base of the first cut. The bud was immediately replaced back into its original location after removing the subtending leaf connected to the bud. Parafilm® Grafting Tape (12 mm x 102 mm) was used to fully cover all cut surfaces, but not the bud itself. The tape was removed after 3 to 4 weeks depending on shoot vigor. Budding was performed weekly from 21 June to 6 Sept. 2006.

Experiment 2: The Effect of Budding Date on Budding Success with Wild Budwood in 2007-08

The effect of budding date on budding success was determined in 2007 using budwood (terminal shoots of current budwood of season with one or more sets of lateral buds and a terminal bud) collected from maple USU-ACGR-1009 in Smithfield Canyon, UT in the Cache Valley National Forest (N 41.874874 W -111.750161). USU-ACGR-1009 is located on a southeast-facing dry slope at an elevation of 2000 m. This maple was selected for bright red fall color and round-pyramidal form (Fig. 12).

Budwood was collected from USU-ACGR-1009, in the morning, on five dates (15 June, 2 July, 16 July, 30 July, and 13 Aug. 2007) based on accessibility and quality. A concerted effort was made to avoid selecting budwood from any natural layers of the tree by collecting only from the main canopy at chest height or higher. The budwood was cut basipetally to the bud scar of 2-year-old wood to avoid damaging the bud and budstick (terminal cutting with two sets of lateral buds (nodes) and a terminal bud). The terminal bud had set and was not actively growing on any of the budsticks. Budwood was cut or broken off the tree, leaves were stripped, and budwood was then sealed in a

plastic freezer bag with a moist paper towel, which was placed inside a similar bag with ice until transported off the mountain. The bags were placed in a cooler with ice (usually after 30 min). The grafting of the buds occurred 2 to 3 h after collection. Only one bud per node could be used due to the small diameter of the budwood.



Fig. 12. Image of bigtooth maple USU-ACGR-1009. The source of wild budwood used for budding experiments.

The coppiced, rootstock plants at the North Logan, UT Greenville Farm were used for budding. All of the rootstocks were vigorously growing due to spring pruning and had not set terminal buds. Ten coppiced seedling maple rootstocks were selected with multiple shoots the same diameter or slightly larger than the scion material. The most vigorous of the wild budwood was selected for budding and only one bud was budded onto one shoot per rootstock. Chip budding (Fig. 13) was used, as previously described in Expt. 1, except the petiole was cut just beyond the bud with the base retained. Buds were placed above the first or second node from the base of the rootstock,

except for those budded on 30 July 2007, which were inadvertently placed between the second and third nodes. Parafilm[®] Grafting Tape was used to cover the entire bud, and left for approximately 6 weeks, and then removed. The above budding process was repeated every 2 weeks with buds placed on different shoots within the same plant.

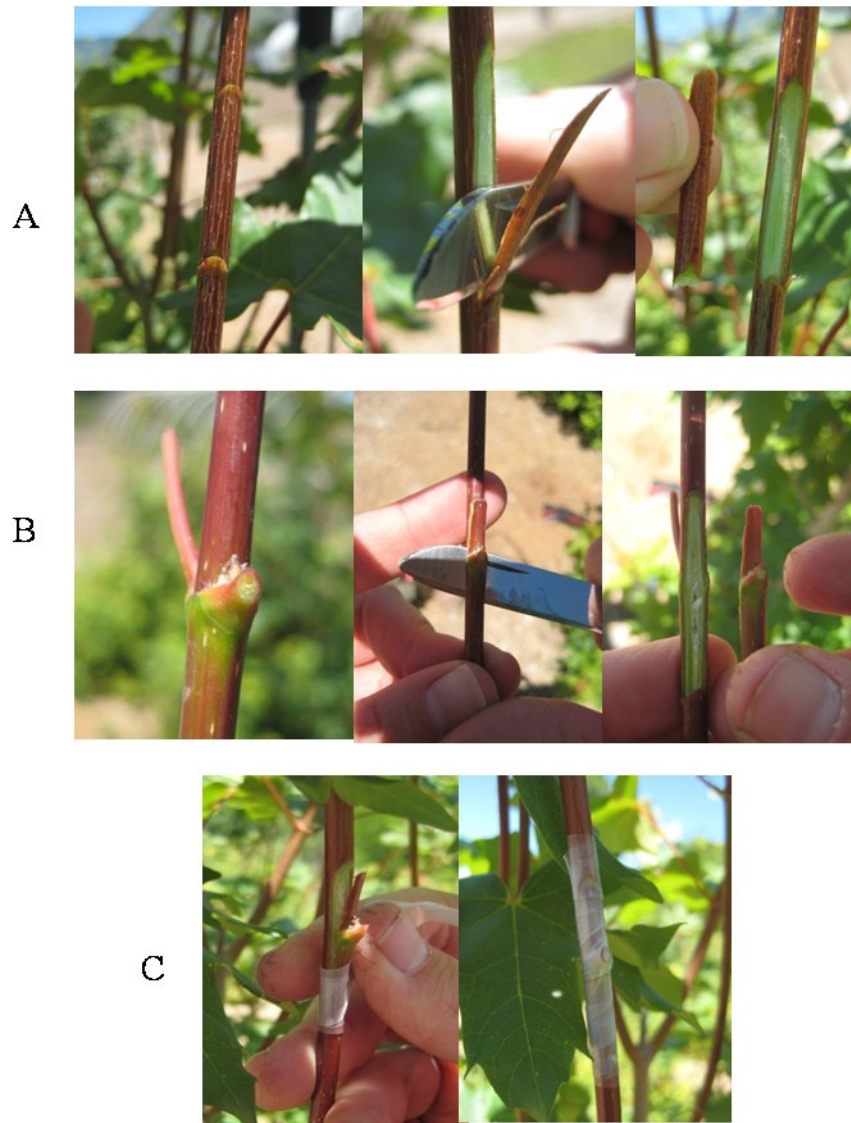


Fig. 13. Images of chip budding process. A). Chip budding the stock plant. B). Chip budding budstick C). Matching bud to stock and taping with Parafilm[®] Grafting Tape.

Experiment 3: The Effect of Budding Date on Budding Success with Wild Budwood in 2009-10

The effect of budding date on budding success using wild budwood was tested again in 2009 with minor changes from the 2007 experiment. A greater emphasis was placed on random pre-selection of all budwood and rootstock shoots. Budwood was collected from the same tree as previously described (USU-ACGR-1009). Budwood was collected in the morning between 7:00 to 9:00 AM. The leaf blades were immediately removed from each budstick. Budsticks were sealed in a plastic freezer bag with a moist paper towel and in a cooler with ice. The grafting of the buds occurred within 3 to 4 h after collection. Budwood was collected in the same manner as Expt. 2, except petioles were left attached to the budstick.

Budding took place on five different dates using randomized budwood. Ninety-six shoots (budsticks) were selected with at least two good nodes and with buds of the required minimum size (full, mature buds on a wide enough stem to permit cutting the stem without damaging the bud). Sixteen budsticks were randomly assigned to each budding date (22 June, 6 July, 20 July, 3 Aug., and 17 Aug. 2009). Budsticks were collected, grouped, and the most vigorous and most basipetal 12 buds were selected for budding. The four remaining budsticks were used as replacements for failed attempts or discarded.

Twelve coppiced rootstocks were randomly chosen, each with five shoots randomly assigned for the different budding dates (22 June, 6 July, 20 July, 3 Aug., and 17 Aug. 2009). Selection of rootstock shoots was based on moderate vigor and uniformity. The 12 selected budsticks were randomly assigned to a shoot within each rootstock. Chip buds were approximately 2 cm in length and placed on the portion of the

rootstock shoot where the internode was the same size or slightly larger than the bud, thus resulting in various locations of the “best fit” along the stem for each bud instead of a predetermined position. Budding was done in the same manner as Expt. 2 except Parafilm® Grafting Tape was used to tie the buds in place. The tape was slit on the opposite side of the bud after 3 weeks if the tape had not split on its own.

Rootstocks were pruned in Apr. 2010 resulting in budded shoots being cut 1 cm above the top of the bud shield. Buds were examined on 10 May 2010 and scored as either alive or dead based on the development of the bud.

Experiment 4: The Effect of Budding Date on Budding Success with Wild Budwood on Containerized Plants 2009-10

The optimal time for budding containerized bigtooth maple seedlings was tested in 2009. Budwood from a wild bigtooth maple specimen in Mantua, UT (USU-ACGR-1022, N 41.50242, W -111.95759) was selected in July and August 2009 to perform an experiment similar to Expt. 3 (Fig. 14).



Fig. 14. Image of USU-ACGR-1022. The source of wild budwood used for budding experiment 4.

Rootstocks were 2-year-old bigtooth maple seedlings planted in 2-gallon pots instead of coppiced, seedlings. Two-year-old maple seedlings were selected for the purpose of saving establishment time in a nursery environment. The seedlings were from the Kaysville Utah Botanical Center and placed under 30% shade cloth at the North Logan, UT Greenville Farm (Fig. 15). The seedlings were watered twice daily for 20 min.



Fig. 15. Image of two-year-old bigtooth maple seedlings in two-gallon containers.

A replicated completely randomized design experiment consisted of the date of bud collection (treatment) and successful bud take (response variable). Budwood was collected from the wild bigtooth maple specimen on five different dates (23 June, 7 July,

21 July, 4 Aug., and 18 Aug. 2009). Eighty budsticks from the main canopy at chest height or higher were previously selected and marked with tape. Sixteen budsticks were randomly selected on each date. The leaves were clipped from the budsticks and the budsticks were transported in sealed plastic freezer bags in a cooler of ice to the North Logan, UT Greenville Farm. Twelve seedling shoots were randomly selected for the twelve randomly-selected budsticks for budding. Chip budding was the budding method performed. Budding was done within 24 h of excising the budwood and usually within 2 to 3 h of collection. A final assessment of budding success was made in the Spring 2010 based on actual budbreak.

Results, Discussion, and Conclusions

Experiment 1: The Effect of Budding Date on “Return Budding” Success in 2006-07

The 2006 buds were evaluated in Spring 2007 and the highest percentage of bud take was in buds grafted in midsummer (28 June to 9 Aug. 2006). The “return budding” bud take average was 51% with 87% at the peak on 28 June and 5 July 2006. Bud take success was reduced in the early summer (21 June 2006) and in the late summer (after 9 Aug.) with the lowest on 6 Sept. 2006 at 0%. The effect of budding date on return budding success was shown to be statistically significant ($p < 0.0001$) for 2006-07 using logistic regression tests of occurrence completed in Statistix 9 (Table B.1 in Appendix B). A test of the differences between least square means was completed in SAS using GENMOD Procedure to show the statistical significance between each individual date. 6 Sept. 2006 was shown to have the greatest significant difference from all of the other dates. 28 June, 5 July, 19 July, and 9 Aug. 2006 were also shown to be significantly

different from 16, 23, and 30 Aug. 2006. 21 June 2006 was significantly different than 28 June and 5 July 2006 (Fig. 16). The 2006-07 data showed that the optimal time for return budding in Utah is late June through early August.

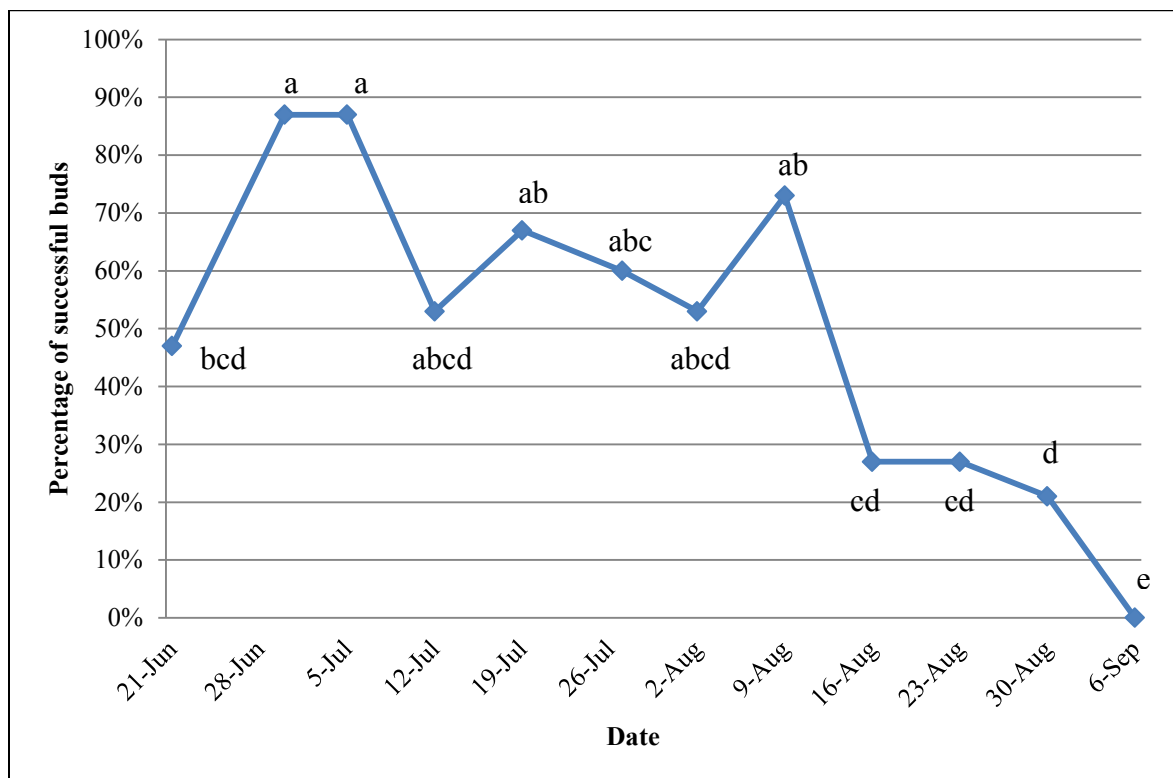


Fig. 16. The effect of 2006 budding dates on the successful bud take of “return buds” on bigtooth maple as measured in Spring 2007. The results showed the overall effect of date to be statistically significant ($p < 0.0001$). Means with the same letter are not significantly different according to the SAS GENMOD Procedure.

Experiment 2: The Effect of Budding Date on Budding Success with Wild Budwood in 2007-08

Successful bud take for 2007 was determined in May 2008. Data showed a longer time period to successfully graft wild buds as compared to grafting “return buds” onto coppiced bigtooth maple seedlings. The results indicated that the highest bud take success came at the end of July (30 July 2007) rather than peaking in early July (Fig. 17).

The results showed mid-June an ineffective time for successful bud take. The wider window of successful bud take was shown from early July to mid-August (30 June, 15 July, and 13 Aug. 2007). Overall, 45% of the buds were successful (59% for July 2007). The effect of budding date on budding success with wild budwood was shown to be statistically significant ($p < 0.045$) for 2007-08 using logistic regression tests of occurrence with Statistix 9 (Table B.2 in Appendix B). The differences of least square means were completed in SAS using GENMOD Procedure to show the statistical significance between dates. 15 June 2007 had significantly lower bud take than all other dates. 30 July 2007 had significantly higher bud take than all other dates. There was no significant difference between 2, 16 July and 13 Aug. 2007.

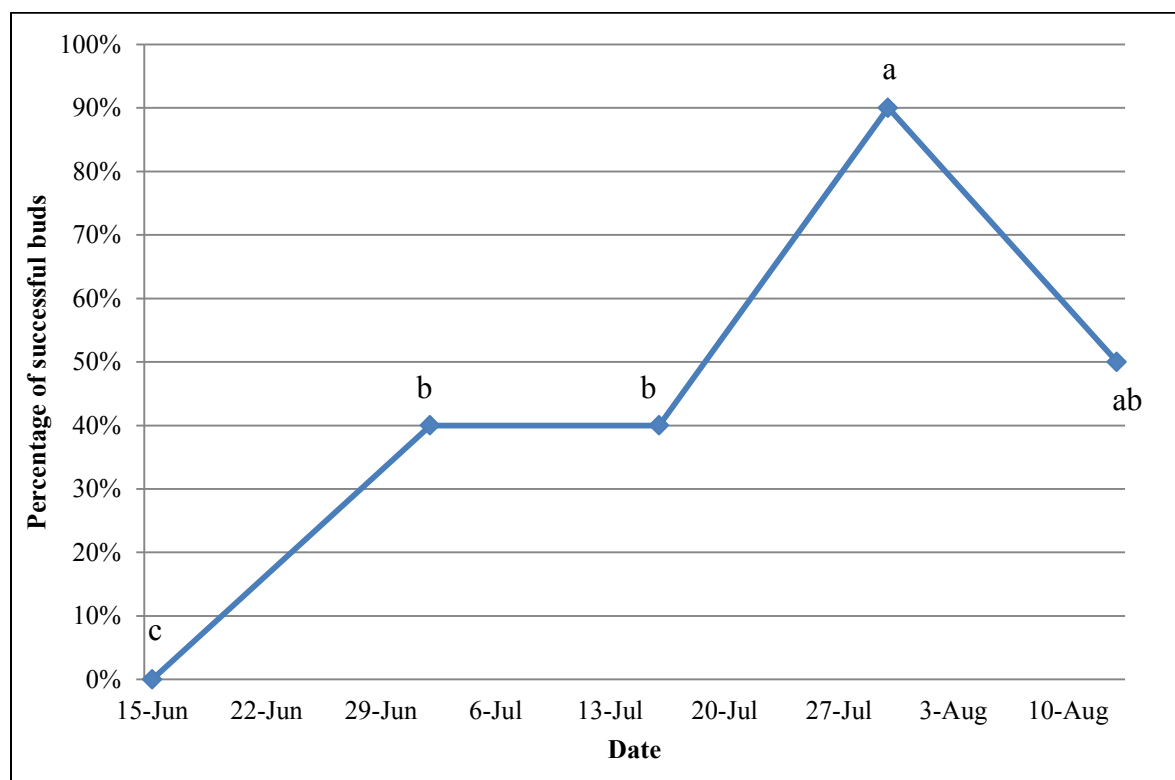


Fig. 17. Effect of 2007 budding dates on successful bud take of wild scion (USU-ACGR-1009) on coppiced seedling rootstocks. The results showed the overall effect of date to be statistically significant ($p = 0.0045$). Means with the same letter are not significantly different according to the SAS GENMOD Procedure.

Some of the rootstock shoots contained aborted buds or damaged buds from the removal of Parafilm[®] Grafting Tape. In efforts with other wild trees (USU-ACGR-1007 and 1008) where budding was more successful (data not shown), the petiole was cut off just above the bud and then left attached to the bud shield. It is possible that leaving the petiole provides needed protection to the bud from the Parafilm[®] Grafting Tape when completely covering the bud.

Experiment 3: The Effect of Budding Date on Budding Success with Wild Budwood in 2009-10

Results showed reduced bud take for earlier budding dates of 22 June (17%) and 6 July 2009 (33%). Success increased dramatically for buds grafted after mid-July through mid-August (20 July, 3 Aug. and 17 Aug. 2009) (Fig. 18). The effect of budding date on budding success with wild bigtooth maple scion was shown to be statistically significant ($p < 0.0001$) for 2009-10 using logistic regression tests of occurrence with Statistix 9 (Table B.3 in Appendix B). The differences in least square means were completed in SAS using GENMOD Procedure. The first two dates (22 June and 6 July 2009) were not shown to be significantly different from each other and there was no significant difference between the last three dates either, but the first two dates and the last three dates were significantly different from each other. The results showed that mid-July through mid-August was the optimal time to bud wild budwood in 2009-10.

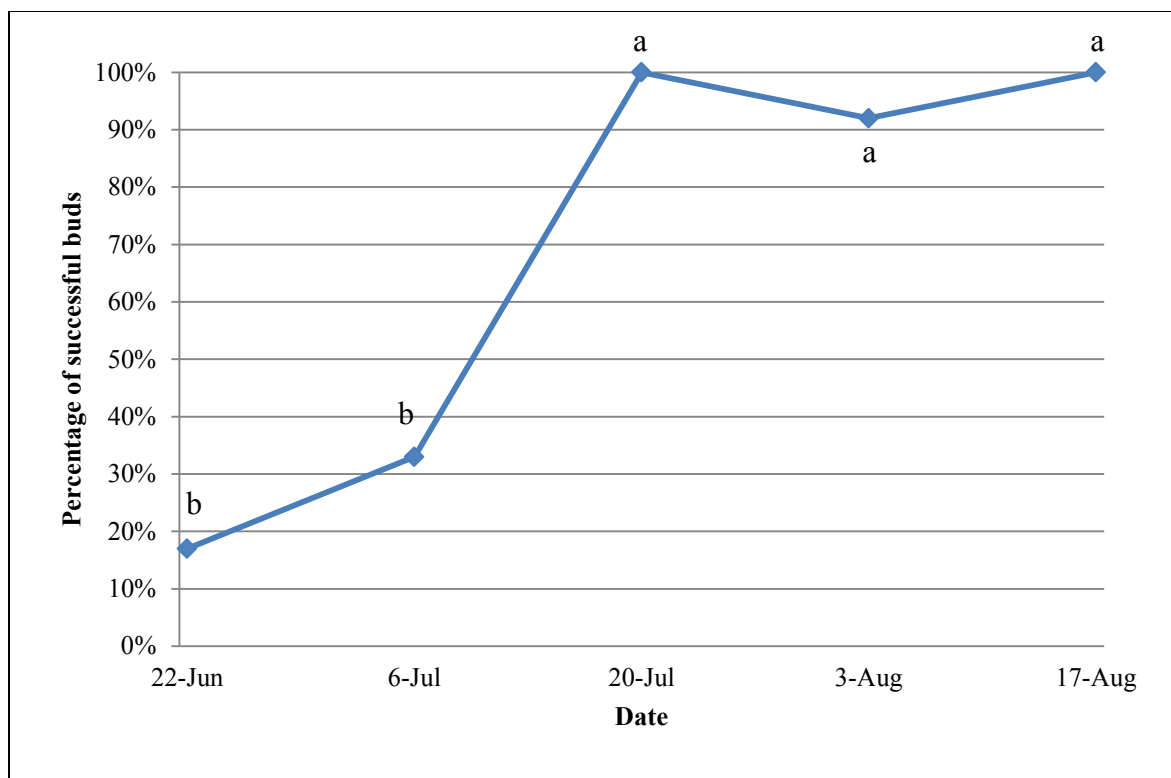


Fig. 18. Effect of 2009 budding dates on budding success of bigtooth maple using wild scion (USU-ACGR-1009). Mid-July to mid-August show the highest bud take success results. The results showed the overall effect of date to be statistically significant ($p < 0.0001$). Means with the same letter are not significantly different according to the SAS GENMOD Procedure.

Experiment 4: The Effect of Budding Date on Budding Success with Wild Budwood on Containerized Plants 2009-10

Data were collected on 14 May 2010 for this experiment. Results showed 4 Aug. 2009 to have the greatest budding success with 46% successful bud take. The lowest was on 23 June 2009 with 0% successful bud take. The highest success rate was in the month of August for wild budwood on the potted plants with an average of 44% successful bud take, whereas the month of July only had an average successful bud take of 13%. The overall bud take average was 23% (Fig. 19). The effect of budding date on budding success with wild budwood on containerized plants was shown to be statistically significant ($p = 0.0077$) in 2009-10 using logistic regression tests of occurrence with

Statistix 9 (Table B.4 in Appendix B). The differences of least square means were completed in SAS using GENMOD Procedure to show the statistical significance between dates. 4 and 19 Aug. 2009 were not significantly different from each other. 23 June and 21 July 2009 were also not significantly different from each other. 7 July 2009 was not significantly different from any of the dates.

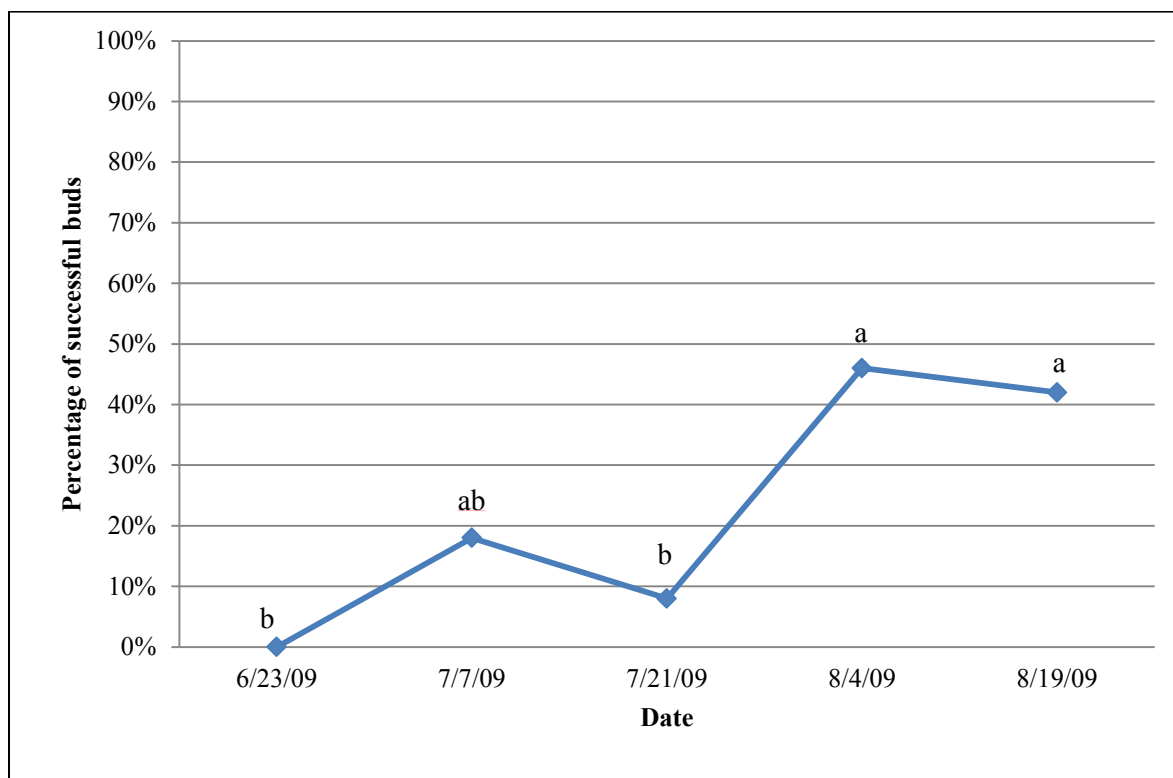


Fig. 19. Effect of 2009 budding dates on budding success of wild scion (USU-ACGR-1022) on containerized two-year-old bigtooth maple. The results showed the overall effect of date to be statistically significant ($p=0.0077$). Means with the same letter are not significantly different according to the SAS Mixed Procedure.

The bud take for this experiment was lower in comparison with Expt. 1-3.

Factors that could have influenced success include the use of container-grown stock plants, 2-year-old seedlings instead of 10-year-old coppiced seedlings, overwintering

conditions (container-grown plants were moved to a cold-frame for the winter), and different levels of budding skills (graduate student and major professor). These factors may have a major impact on successful budding in Utah.

Our objective was met by finding an ideal time range to propagate bigtooth maple via budding. A great deal of variation remains in the budding results despite the efforts to reduce the impact of season, budding skills, and other factors affecting budding success. General time periods can be identified even though no single date can be identified as optimum. Figure 20 shows the budding success of the bud dates using logistic regression statistical analyses from 2006, 2007, and 2009 (Expt. 1-3).

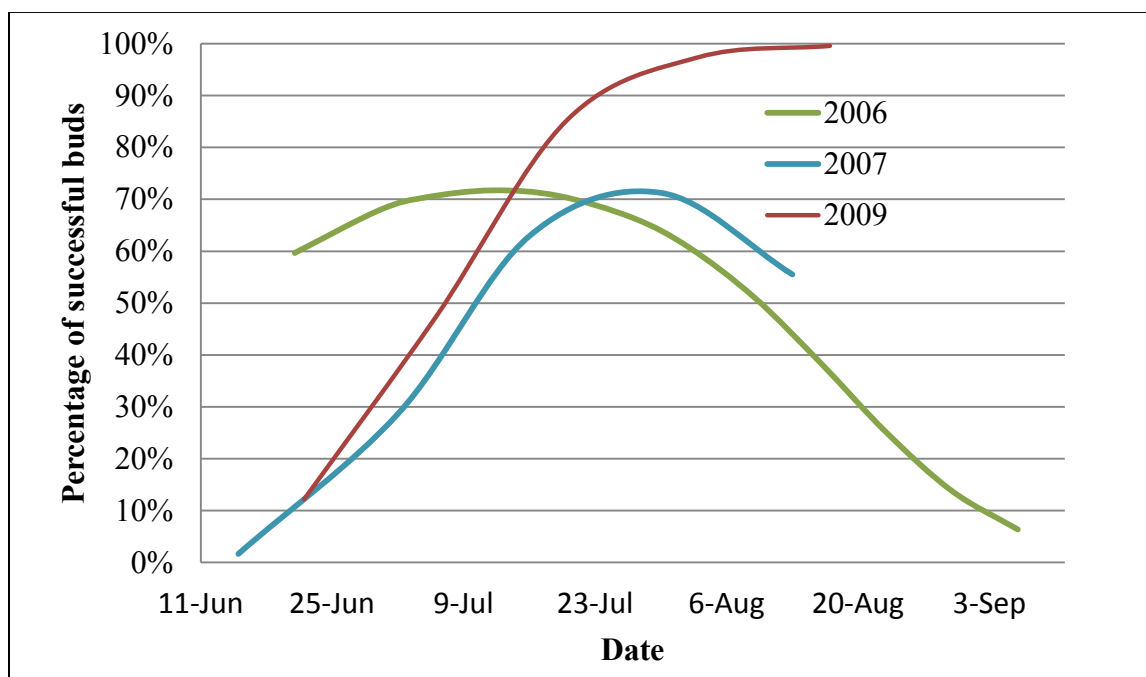


Fig. 20. Effect of 2006, 2007, and 2009 budding dates on successful budding for bigtooth maple (Expts. 1-3) using logistic regression statistical analyses. Each year shows a different trend.

Figure 21 shows the budding success on 2-year-old, containerized bigtooth maple seedlings. From the figures we can conclude that the budding dates from July through mid-August produced acceptable bud take success. This defined window allows us to successfully target wild budwood collection to certain dates with the greatest potential for budding success in bigtooth maple.

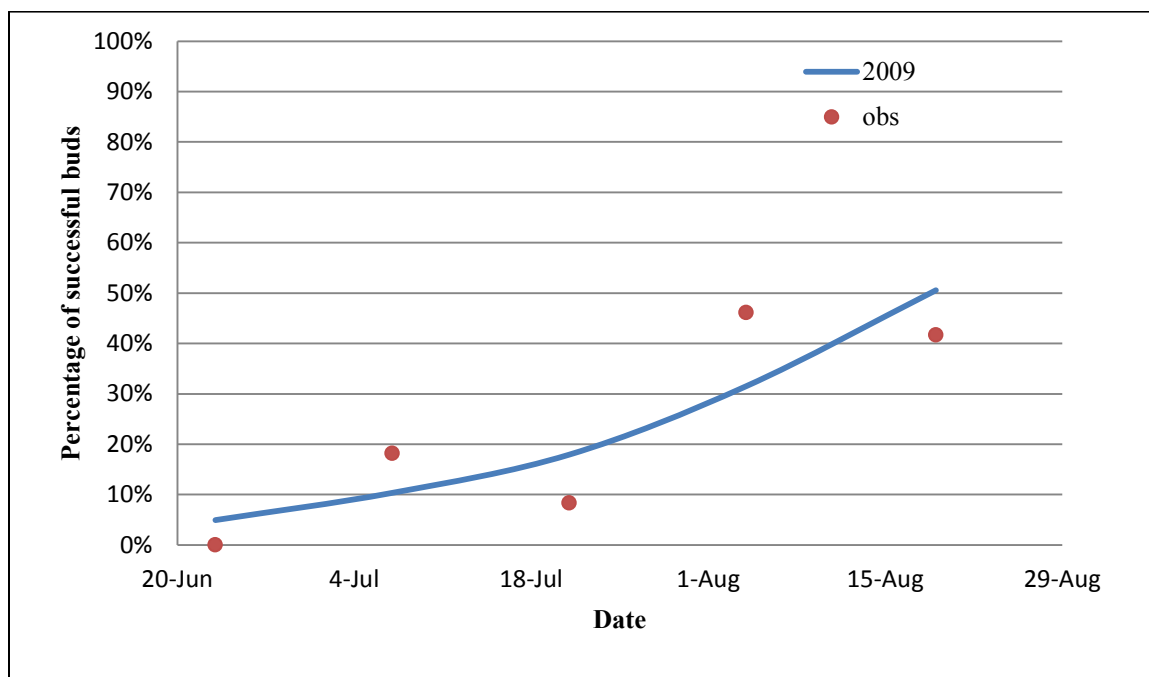


Fig. 21. Effect of 2009 budding dates on budding success on 2-year-old, containerized bigtooth maple (predicted and actual outcomes). An increasing trend is shown with a large increase from mid-July to beginning August.

Future Work

Propagation and production experiments may be carried out in the future to optimize other propagation techniques such as cuttings and layering. More research may be conducted on the overwintering aspect of the growth period. For example, relevant research questions are: Why is there an apparent winter-kill of grafted buds? How much

effect does the native environmental climate have on the bud when brought into a nursery setting? Does it cause potential water stress? Why do coppiced maple rootstocks result in higher percentages of bud take than containerized 2-year-old seedling rootstock?

These are just a few of the questions that merit further research.

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CHAPTER IV

EFFECT OF ETIOLATION ON ROOTING BIGTOOTH MAPLE

(*ACER GRANDIDENTATUM* NUTT.) CUTTINGS

ABSTRACT

Adventitious root formation in bigtooth maple (*Acer grandidentatum* Nutt.) was significantly increased by etiolation of softwood cuttings. Six selections of wild bigtooth maple from Cache County, UT were grafted onto seedling rootstocks growing in a stoolbed environment and used as stock plants. Stock plants were severely pruned just below the third node from the base in the dormant season of 2009 and 2010. Open-ended, black, velour, drawstring bags were placed over the end of pruned shoots at bud swell allowing new shoots to develop and grow out the end of the bag while maintaining etiolation of the shoot base. The cuttings were harvested after 3 to 4 weeks, trimmed to two nodes above the base, wounded, and the bases dipped for 5 seconds in 4000 ppm indole-3-butyric acid/ 2000 ppm naphthaleneacetic acid (Dip 'N Grow[®]). Cuttings were stuck in a pre-moistened 3:1 perlite:peat rooting medium and placed on heating mats (20-30 °C) under an intermittent mist system (7 seconds of mist every 12 minutes) using reverse osmosis water. Rooting was evaluated on the cuttings after 4 weeks. Results indicated that 89% (2009) and 85% (2010) of the etiolated cuttings rooted and only 47% (2009) and 17% (2010) of the non-etiolated cuttings rooted. Etiolated cuttings produced on average 11.3 (2009) and 7.2 (2010) roots per cutting and the non-etiolated cuttings produced on average 2.1 (2009) and 0.5 (2010) roots per cutting. We conclude that

propagation of bigtooth maple is significantly improved through etiolation of softwood cuttings.

Introduction

Bigtooth, or canyon maple, (*Acer grandidentatum* Nutt.) is a small tree native to the Intermountain West and possesses superior characteristics valued by the nursery and landscape industries (Barker, 1974; Kuhns, 2003; Tankersley and Emino, 1981). Bigtooth maple is cold hardy (-35 to -38 °C) (Kuhns, 2003), drought tolerant (38 to 51 cm of annual precipitation), and adapts well to a wide range of soils and habitats. The leaves are simple, tooth-like, and deeply-lobed. Foliage colors such as reds, oranges, and yellows emerge in the fall (Barker, 1974, 1977). Genetic diversity among bigtooth maple causes different canopy forms, such as columnar, pyramidal, and round. The above bigtooth maple characteristics will satisfy the demands of the nursery and landscape industries via clonal propagation.

Past efforts in clonal propagation of bigtooth maple have resulted in little success (Bowen-O'Connor et al., 2007; Tankersley, 1981). Mound layering of bigtooth maple resulted in 20% rooting and French layering 71% rooting (Tankersley, 1981). Although French layering had a higher rooting percentage, it is not an economical propagation practice. Micropropagation of bigtooth maple resulted in 15% rooting (Bowen-O'Connor et al., 2007) and is not a viable means of cloning mature specimens. Finally, stem cuttings resulted in 0.8% rooting success (Tankersley, 1981). Methods of clonal propagation must be improved to satisfy the high demands of the nursery and landscape industries (Bowen-O'Connor et al., 2007).

Research demonstrates that the combination of juvenile cuttings, auxins, and etiolation (exclusion of light) are undeniable assets in improving propagation in hard-to-root woody species (Griffin and Bassuk, 1996; Herman and Hess, 1963; Maynard and Bassuk, 1987). No concrete explanation exists as to why juvenile plants initiate adventitious rooting better than non-juvenile plants, but theories suggest factors such as preformed root initials and stem anatomy have an effect on adventitious rooting (Clark, 1982). The relationship between etiolation and adventitious rooting is another propagation technique that is not completely understood (Maynard and Bassuk, 1987). Researchers have discovered that etiolated stem tissue is less lignified, possesses stem primordia, lacks mechanical properties, has a higher content of auxin, and is more sensitive to auxin than non-etiolated tissue (Frolich, 1961; Gardner, 1936; Herman and Hess, 1963; Maynard and Bassuk, 1987; Reid, 1922). Adventitious root formation and uniformity are increased in hard-to-root cuttings when wounded and treated with an auxin, such as IBA (indole-3-butyric acid) (Alsup et al., 2003; Griffin and Bassuk, 1996). Propagation via cuttings is a cheap, simple, and effective method that will aid the nursery and landscape industries in cloning ideal bigtooth maple specimens (Griffin and Bassuk, 1996). We hypothesize that bigtooth maple can be successfully propagated using etiolated, auxin-treated cuttings and will result in higher rooting percentages than past experiments.

Materials and Methods

In Summer 2009 and 2010, we used five to six (depending on the year) selections of bigtooth maple (USU-ACGR-1001, 1002, 1003, 1004, 1005, and 1009) for the

softwood cutting propagation experiment. The above trees were selected from the wild and grafted onto seedling rootstocks growing in a coppiced, stoolbed environment (North Logan, UT Greenville Farm). Preparations for the experiment began in late January and early February. We made pruning cuts immediately below the third bud from the bottom (discrediting any bud less than 13 mm from bottom) leaving a 7 to 13 cm stub above the most acropetal bud.

The etiolation process began when we placed open-ended, black, velour, drawstring bags over the pruned, terminal shoots. The drawstring was tied just below the uppermost buds. The part of the pruned shoot, located above the bud, supported and prevented the bag from folding over and inhibiting growth of the cutting. We placed the bags over the pruned shoots at incipient bud swell on three different dates in 2009 (22 and 28 Apr., and 2 May), and on four different dates in 2010 (20, 21, and 26 Apr., and 8 May) depending on the selection. The buds broke and the shoots developed in the reduced light of the bag environment. The shoots emerged from the bag, acclimating to full sun, and the etiolated base of the shoot remained covered by the bag (Fig. 22A). Pruned shoots were randomly assigned to be either etiolated or non-etiolated (control).

We harvested the softwood cuttings when two sets of fully expanded leaves emerged from the top of the bag (in early June after approximately 3 to 4 weeks of shoot elongation), and when the total shoot length of non-etiolated shoots averaged around 20 to 25 cm (Fig. 22B). We harvested the cuttings on three different dates in 2009 (21, 22, and 28 May) and on four different dates in 2010 (24, 31 May, 2 and 7 June). The time of harvest was between 6:00 to 8:00 AM. The procedure consisted of cutting the shoot

below the drawstring of the bag. Cuttings were immediately sealed in 9.46 L plastic freezer bags and held in an ice-filled cooler until stored in a refrigerator (4 °C).



Fig. 22. Images of bigtooth maple trees at the beginning and harvest periods of the etiolated cuttings experiment. A) Pruning cuts shown with 7 to 13 cm stub. Black, velour bags tied with drawstring immediately above uppermost bud is used to promote etiolation. The bags were cut at the top to allow shoots to grow outward, yet dark enough to cause etiolated shoots. B) Softwood cuttings were collected when two sets of fully expanded leaves had emerged from the top of the bag and the average length of non-etiolated shoots was 20 to 25 cm (after approximately 3 to 4 weeks around early June).

We randomly selected each harvested shoot from the freezer bag after removal from the refrigerator. Each harvested shoot consisted of two cuttings due to the opposite branching of bigtooth maples. We selected only one cutting for sticking using the following criteria: 1) If the cuttings were equally healthy, then a coin was flipped and one cutting was randomly selected; 2) If only one cutting was healthy, then that healthy one was chosen; and 3) If both cuttings were missing, too small, or too damaged, then neither

cutting was used. The selected cutting was cut immediately above any latent buds at the base and above the second node resulting in two sets of leaves on each cutting. We recorded data on the diameter (mm) of the cutting, diameter of the shoot (at the point where the cutting emerged), leaf damage (insect or heat damage), number of leaves per cutting, length of cutting before cut (from base to apical bud), length of cutting after cut (from final base cut to second node (cm)), and etiolation effectiveness based on visual appearance (judged to have effective etiolation if the base was a lighter green color than control).

We wounded the cutting on one (2010) or two (2009) sides (depending on the year) by scraping 1 cm of the bark down to the woody tissue with a knife held perpendicular to the stem (Fig. 23A). We sanitized the knife in 70% ethyl alcohol between each cutting. The cuttings were dipped in 4000 ppm IBA (indole-3-butyric acid) and 2000 ppm NAA (naphthaleneacetic acid) as Dip 'N Grow[®] (EPA Reg. #64388-1) (20 mL Dip 'N Grow[®] (DNG) (1% IBA and 0.5% NAA) diluted with 30 mL of 50% ethyl alcohol) for 5 s and shaken once to remove the excess.

We stuck the cuttings approximately 2 to 5 cm deep in every other cell of a 606 tray (63.5 mm x 63.5 mm x 76.2 mm cells) in pre-moistened media (3:1 perlite:peat mixture) (Fig. 23B). We misted the cuttings with deionized water using a generic spray bottle to keep the cuttings hydrated before placing them on the mist bench.

We shaded the greenhouse with Kool Ray[™] liquid shade in 2009. The cuttings were also placed under double-folded Reemay[®] shade cloth, which resulted in 350 to 470 $\mu\text{mol PPF m}^{-2} \text{s}^{-1}$ of light (Fig. 24A). The Reemay[®] reduced air movement from cooling fans and decreased light levels. The greenhouse was covered with black, plastic shade

cloth at 60% shading in 2010 to improve consistency in shading, which resulted in similar light levels of $\sim 470 \mu\text{mol PPF m}^{-2} \text{s}^{-1}$ (Fig. 24B). We used one layer of Reemay[®] shade cloth instead of two, and no shade cloth above the cuttings to aid against excessive air movement within the greenhouse.

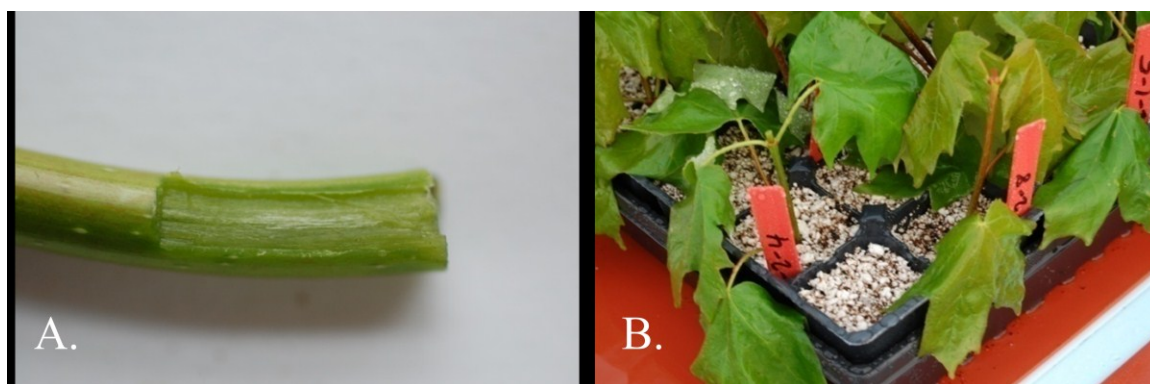


Fig. 23. Image of wounded bigtooth maple etiolated cutting and the cells wherein the cuttings were stuck. A) Each cutting was wounded as deep as the xylem tissue (1 cm in length). B) Each cutting was stuck in every other cell (approximately 2 to 5 cm in depth in 63.5 mm x 63.5 x 76.2 mm cells) in pre-moistened media (3:1 perlite:peat mixture).



Fig. 24. Images of different shading methods for bigtooth maple cuttings in 2009-10. A) Kool Ray[™] liquid shade and double-folded Reemay[®] shade cloth were used to reach light levels of 350 to 470 $\mu\text{mol PPF m}^{-2} \text{s}^{-1}$ B) Black, woven, plastic 60% shade cloth was used in 2010 with no need for double-folded Reemay[®] shade cloth resulting in similar light levels (350 to 470 $\mu\text{mol PPF m}^{-2} \text{s}^{-1}$).

We placed the cuttings on heating mats with the temperature set at 25-26 °C, but with actual bottom heat ranging from 20 to 30 °C. Misting was done with reverse osmosis treated water (Fig. 25). The mist was applied at 7 s every 12 min from 6:00 AM to 9:00 PM. The greenhouse day/night temperatures were set at 21 °C day/15.5 °C night, but ranged from 19 °C to 22.5 °C throughout the day.



Fig. 25. Image of growing conditions of bigtooth maple cuttings. The cuttings were placed on heating mats with bottom heat at 20 to 30 °C. Misting was done with reverse osmosis-treated water with an intermittent mist system (7 s/ 12 min).

Disease and root rot were common among the bigtooth maple cuttings in preliminary experiments. *Botrytis* was prevented by applying weekly applications of Cleary 3336™ at 1 mL/L. Root rot was prevented by applying a one-time drench of Mefenoxam 2AQ at a rate of 0.6 mL/3.8 L (17 mL/cell) after sticking. The cuttings were

randomly rotated every other day and any dead or diseased leaves were removed so as to reduce potential sources of inoculums.

We recorded rooting response of the cuttings after 4 weeks. We removed the flats of cuttings from the propagation bench. We gently removed the cuttings from the rooting medium and dipped the roots of the cuttings in water to detach the excess media. We evaluated the rooting on each cutting according to these criteria:

- Presence of roots
- Number of roots per cutting
- Length of an average root (2009) Estimated average length of all roots (2010)
- Number of leaves remaining
- Callus presence (yes or no)

We defined a root as anything longer than it was wide (usually ~1 mm). Root count was conducted in a non-destructive manner in 2009. In 2010, root count was conducted in a destructive manner by removing all roots to insure greater accuracy. We counted each individual root. We recorded the number of leaves remaining on the cutting and the extent of root branching. Digital photographs of all cuttings were also taken. The rooting data was analyzed with the statistical programs, Statistix 9 and SAS 9.2, as follows:

A two-sample t-test was completed to determine if etiolation had any effect on the percentage of rooted cuttings in 2009 and 2010. We performed a square root transformation on the percentage for the analyses to better fit the t-test normality assumption. We used the Satterthwaite method due to unequal variances. A Mixed Procedure was completed using SAS to find the significant differences of least square

means between trees and rooting percentage. A two-way ANOVA (analysis of variance) was completed to determine the effect of etiolation on the number of roots. We performed a log transformation on the number of roots to fit the normality assumption. A Mixed Procedure was completed using SAS to find the significant differences of least square means between trees and number of roots. A chi-square test for heterogeneity or independence was completed to determine the effect of etiolation on leaf damage. Leaf damage observations were made when we harvested the cuttings. An ANOVA was carried out to determine the effects of etiolation on the number of leaves lost. The number of leaves lost was observed during the rooting process of the cuttings. A chi-square test of heterogeneity or independence was also performed to determine the effect of etiolation on callus.

Results

The effect of etiolation on percentage of rooted cuttings was evaluated. In 2009, 89% of the etiolated cuttings rooted, as compared to 47% of the non-etiolated (Fig. 26). Etiolated cuttings of USU-ACGR-1001, 1002, 1004, and 1005 all had over 83% rooting (Tables C.1, C.2, and C.3 in Appendix C). USU-ACGR-1005 had the highest rooting percentage at 100% and USU-ACGR-1003 had the lowest rooting percentage at 71% of etiolated cuttings. The statistical analyses showed that the overall percentage of rooting on etiolated cuttings was significantly greater ($p= 0.0004$) than non-etiolated cuttings. The SAS Mixed Procedure showed the percentage of rooting on etiolated cuttings from USU-ACGR-1004 and -1005 to be significantly higher than all non-etiolated cuttings.

The percentage of rooting on non-etiolated cuttings from USU-ACGR-1001 and -1003 was shown to be significantly lower than all etiolated cuttings.

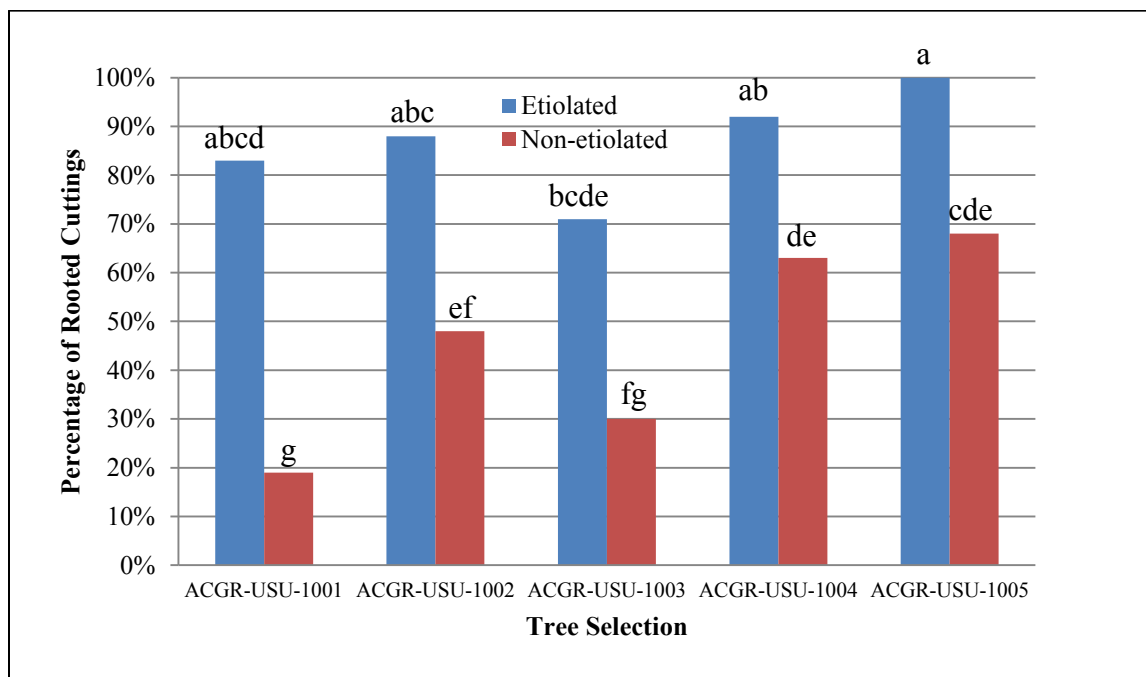


Fig. 26. The effect of etiolation in 2009 on the percentage of rooted cuttings in bigtooth maple. Etiolation significantly increased the percentage of rooted cuttings in all clones as shown by the two-sample t-test. Means with the same letter are not significantly different according to the SAS Mixed Procedure.

Etiolated cuttings had an average of 85% rooting success in 2010, whereas only 17% of the non-etiolated cuttings formed roots (Table C.4 in Appendix C). The highest rooting percentage in 2010 was USU-ACGR-1005, which was also the highest in 2009, with a 95% rooting success. The highest rooting percentage for non-etiolated cuttings was USU-ACGR-1004 with 36% rooted cuttings. The statistical analyses showed that in 2010 the percentage of rooting on etiolated cuttings was significantly greater than non-etiolated cuttings ($p=0.0000$) (Fig. 27).

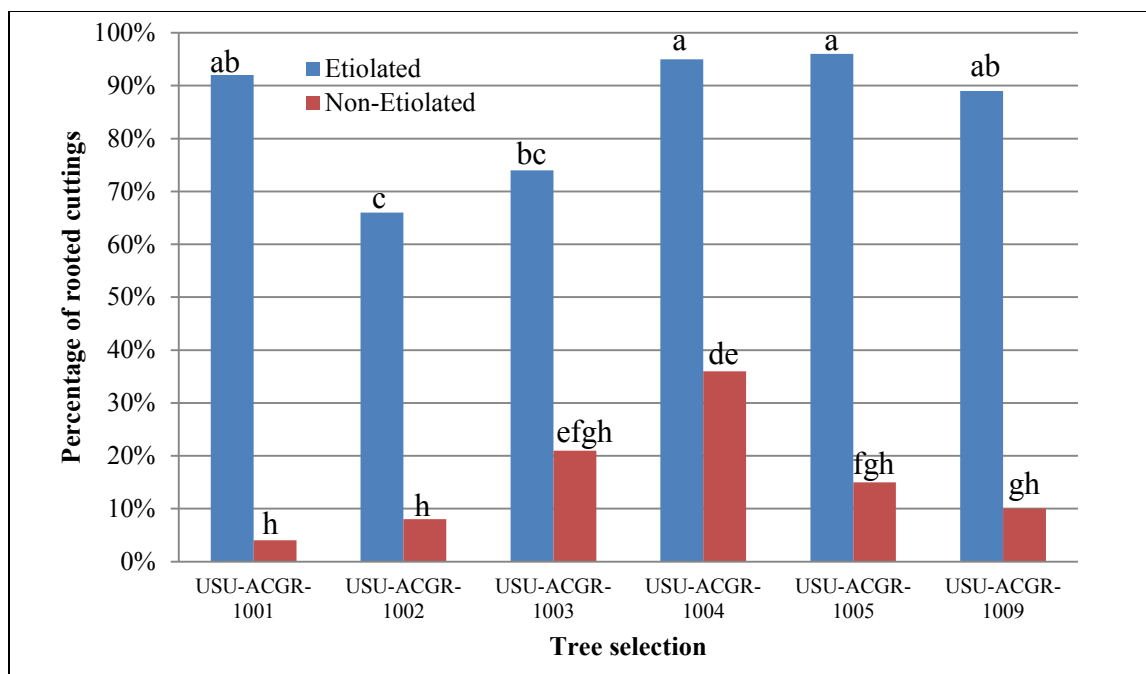


Fig. 27. The effect of etiolation in 2010 on the percentage of rooted cuttings of bigtooth maple. Etiolation significantly increased the percentage of rooted cuttings in all clones as shown by the two-sample t-test. Means with the same letter are not significantly different according to the SAS Mixed Procedure.

Data collected on the effect of etiolation on number of roots per cutting indicated that etiolated cuttings had an average of 11.3 roots per cutting, while non-etiolated averaged 2.1 roots per cutting in 2009 (Table C.5, C.6, and C.7 in Appendix C). Statistical analyses showed that cuttings from all tree selections had significantly more roots per cutting when etiolated. The highest average number of roots per etiolated cutting was on USU-ACGR-1005 with 17.6 roots per cutting and the lowest was on USU-ACGR-1003 with 3.3 roots per cutting (Fig. 28). The highest average number of roots for the non-etiolated cuttings was on USU-ACGR-1004 with 3.6 roots per cutting and the lowest was on USU-ACGR-1001 with 0.8 roots per cutting. The number of roots on etiolated cuttings was significantly greater than the number of roots on non-etiolated cuttings ($p < 0.0000$).

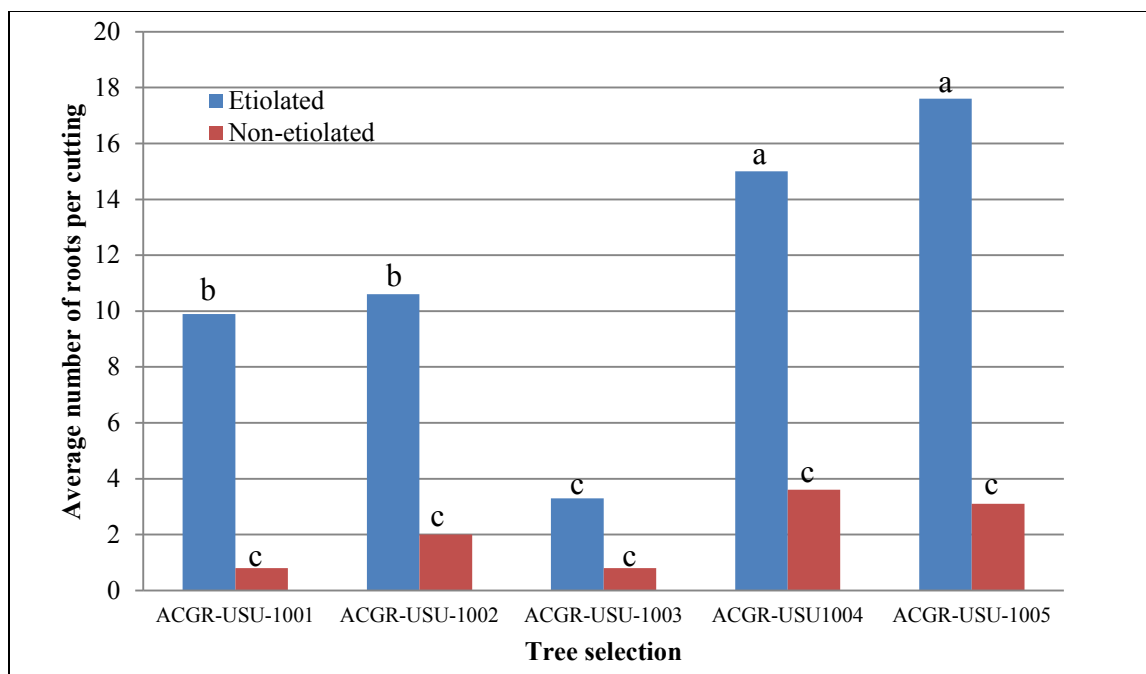


Fig. 28. Effect of etiolation in 2009 on average number of roots per cutting of bigtooth maple. The number of roots on etiolated cuttings was significantly greater than the number of roots on non-etiolated cuttings ($p < 0.0000$). Means with the same letter are not significantly different according to the SAS Mixed Procedure.

The number of roots per cutting in 2010 averaged 7.2 roots per cutting on etiolated shoots and 0.5 roots per cutting on the non-etiolated shoots, which is much lower than 2009 (Table C.8 in Appendix C). The maximum number of roots for non-etiolated cuttings was 9.0 roots per cutting and for the etiolated cuttings was 25.0 roots per cutting. Etiolated cuttings from USU-ACGR-1004 had the highest average of 11.1 roots per cutting and the lowest average was USU-ACGR-1003 with 3.2 roots per cutting. Non-etiolated cuttings from USU-ACGR-1004 had an average of 1.1 roots per cutting, and all the other tree selections had less than one for the average number of roots per non-etiolated cutting (Fig. 29). The analyses showed that etiolated cuttings had a significantly greater number of roots per cutting than non-etiolated cuttings with a $p\text{-value} < 0.0000$.

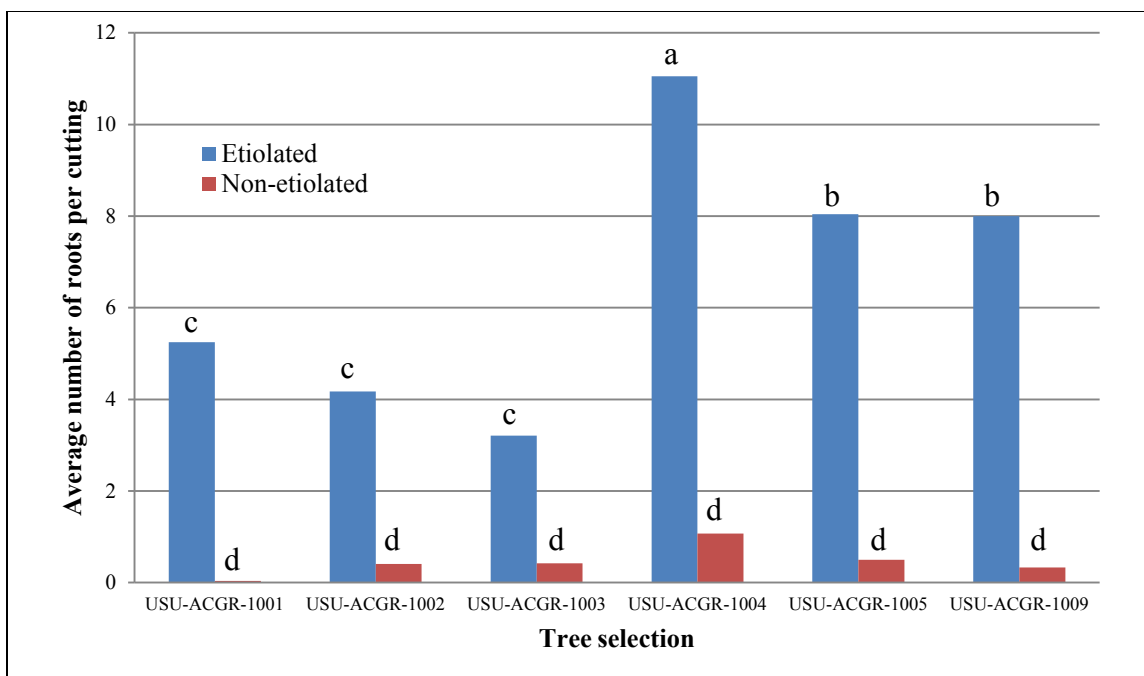


Fig. 29. Effect of etiolation in 2010 on average number of roots per cutting of bigtooth maple. The analyses showed that etiolated cuttings had a significantly greater number of roots per cutting than non-etiolated cuttings with a p -value <0.0000 . Means with the same letter are not significantly different according to the SAS Mixed Procedure.

We observed a higher quantity of leaf damage among the etiolated cuttings than the non-etiolated cuttings at the time of harvest (Table C.9 in Appendix C). In 2009, 93% of the leaves of the etiolated cuttings were damaged compared to 33% of the leaves on non-etiolated cuttings. The statistical analyses showed that leaf damage on etiolated cuttings was significantly higher than the leaf damage on non-etiolated cuttings (p -value <0.0000).

Similar results were manifested among the 2010 data (Table C.10 in Appendix C). Ninety percent of the leaves from the etiolated cuttings were damaged and only 34% of the leaves were damaged from the non-etiolated cuttings. The chi-square test showed a strong association (p -value <0.0000) between etiolation and leaf damage.

We observed that many leaves senesced during the rooting time period. We also observed that the etiolated cuttings had lost more leaves than the non-etiolated cuttings by the time of root count. Statistical analyses were completed to show the effect of etiolation on number of leaves lost. The leaves lost averaged 1.35 leaves per etiolated cutting and 0.68 leaves per non-etiolated cutting in 2009 (Fig. 30).

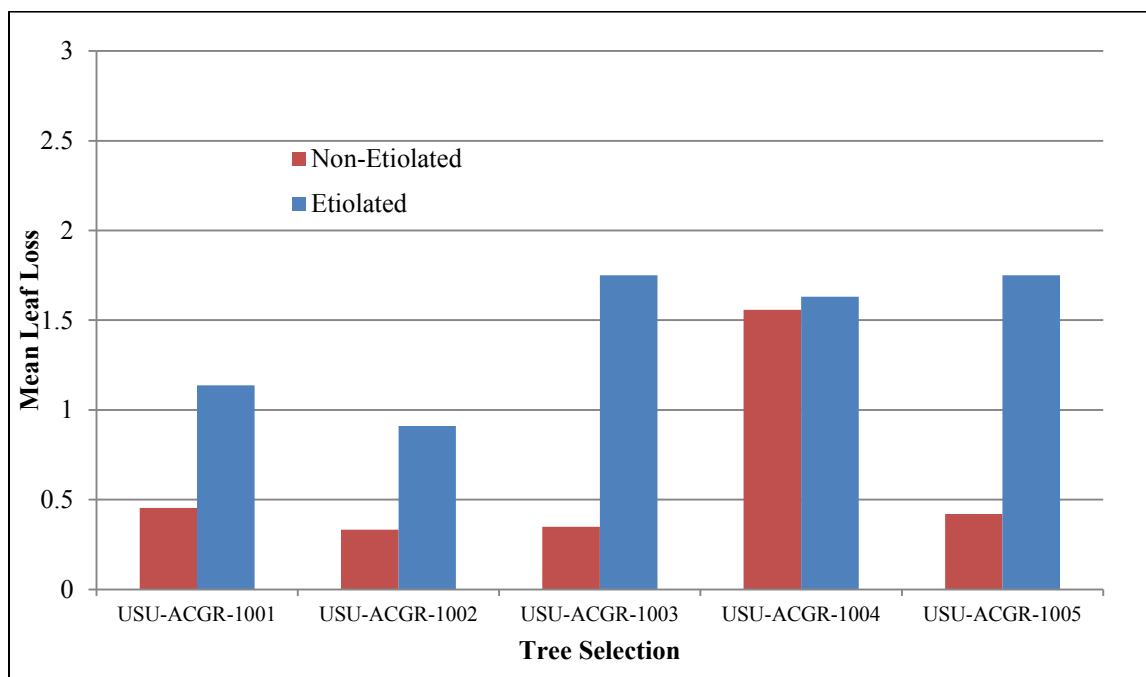


Fig. 30 Effect of etiolation in 2009 on number of leaves lost of bigtooth maple. Statistical analyses showed that etiolated cuttings had a greater overall leaf loss than the non-etiolated cuttings.

In 2010, the average number of leaves lost was 1.89 leaves per etiolated cutting and 0.98 leaves per non-etiolated cutting (Fig. 31). An ANOVA on number of leaves lost on etiolated and non-etiolated cuttings was carried out. The results of the analyses showed that etiolated cuttings had an overall significantly greater leaf loss than non-etiolated cuttings for 2009-10 ($p < 0.0000$) (Table C.11 and C.12 in Appendix C).

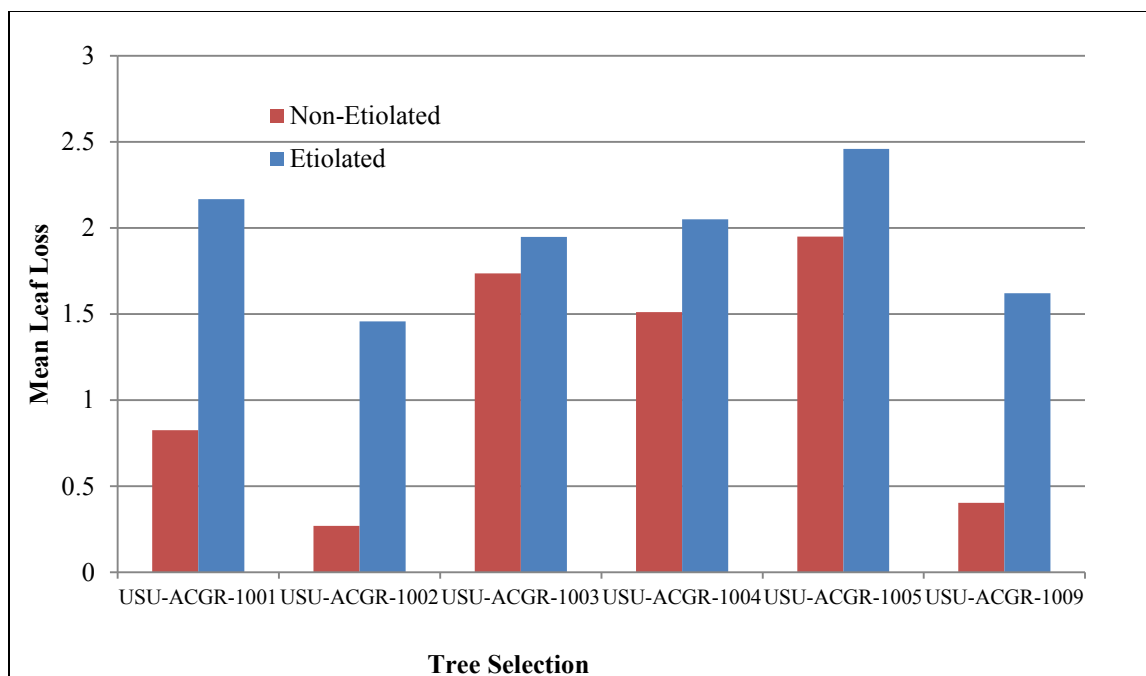


Fig. 31. Effect of etiolation in 2010 on number of leaves lost of bigtooth maple. Statistical analyses showed that etiolated cuttings had a greater overall leaf loss than the non-etiolated cuttings.

We observed that many of the etiolated and non-etiolated cuttings produced callus at the time of root count. Statistical analyses were completed to analyze the effect of etiolation on callus. In 2009, 100% of the etiolated cuttings and 94% of the non-etiolated cuttings developed callus. Comparatively, in 2010, 98% of the etiolated cuttings and 75% of the non-etiolated cuttings developed callus. We completed a chi-square test of heterogeneity or independence (Table C.13 and C.14 in Appendix C) and the etiolated cuttings from 2009 and 2010 were shown to have significantly overall greater callus development than non-etiolated cuttings (2009: $p=0.0022$ and 2010: $p<0.0000$).

Discussion and Conclusions

The results clearly showed that etiolation had a significant effect on rooting. The greatest difference among etiolated and non-etiolated cuttings in 2009 was found with USU-ACGR-1001 where etiolation increased the rooting percentage by 64 percentage points. The least difference among etiolated and non-etiolated cuttings in 2009 was with USU-ACGR-1004 where etiolation increased rooting percentage by 29 percentage points. The rooting percentages for the etiolated cuttings had closer results between clones and less variation than the non-etiolated cuttings. The percentage of rooting among the non-etiolated shoots had a difference of 30 percentage points from 2009 (43% non-etiolated rooted) to 2010 (17% non-etiolated rooted). USU-ACGR-1001 had the greatest difference in 2010 in the rooting percentage between treatments from 92% etiolated rooting success to 4% non-etiolated rooting success (88 percentage points). USU-ACGR-1003 had the least difference in 2010 (53 percentage points from 74% etiolated rooting success to 21% non-etiolated rooting success). We can conclude that etiolated bigtooth maple cuttings had a significantly higher rooting percentage than non-etiolated cuttings.

The results clearly showed that etiolation also had a significant effect on the number of roots per cutting. The data were consistent both years with etiolated cuttings having an overall higher average number of roots per cutting than the non-etiolated cuttings. The 2010 data indicated a lower number of roots per cutting than 2009 on both etiolated and non-etiolated cuttings. We observed the number of roots per cutting in USU-ACGR-1001 and USU-ACGR-1002 in 2010 were 50% less than the number of roots per cutting in 2009. A factor may be that USU-ACGR-1001 and 1002 were harvested

physiologically earlier in 2010. However, USU-ACGR-1004 and 1005 were also lower in 2010 compared to 2009, but were not harvested physiologically earlier than the USU-ACGR-1004 and USU-ACGR-1005 cuttings in 2009. USU-ACGR-1003 was the only tree that had consistently the same number of roots per cutting in both years. Obviously, other unknown factors contributed to these differences in number of roots per cutting and it may be a normal factor such as variations in weather from year to year. The main point to conclude is that etiolated cuttings had a significantly greater number of roots per cutting than non-etiolated cuttings.

Both the 2009 and 2010 data illustrate that etiolation treatments result in leaf damage prior to the harvest of the shoots. We are unable to conclude from this research if the damage was a physiological response to etiolation or if the damage was due to the method of etiolation used (new shoots elongating through black, velour bags). Some damage appeared to be from heat and one can assume that the temperature inside the black bags was warmer than found outside in the open air. Further damage was caused by paper wasps (*Polistes metricus*) nesting in the bags. We are unable to conclude that leaf damage on bigtooth maple cuttings is a serious problem in successfully establishing bigtooth maple cuttings. We can conclude that etiolation, or rather, the method of etiolation used (new shoots elongating through black, velour bags) had an increased effect on leaf damage among the bigtooth maple cuttings.

The data also showed that etiolated cuttings had an overall significantly greater leaf loss than the non-etiolated cuttings. This leaf loss may have been due to natural physiological reasons, such as dropping leaves to use the needed energy for rooting. Another factor may have been that the leaves on the etiolated cuttings were too damaged

and dropped. We also observed from the data that the 2010 cuttings had a greater average leaf loss than 2009. We can conclude that etiolated cuttings showed an overall significantly greater leaf loss than the non-etiolated cuttings even though the reasons for leaf loss in bigtooth maple cuttings were unknown.

We mentally hypothesized that etiolated cuttings would have more callus development than non-etiolated cuttings because etiolated cuttings have a higher rooting success than non-etiolated cuttings. We observed that callus formation was present on a large number of both etiolated and non-etiolated bigtooth maple cuttings. The development of callus in cuttings is associated with the healing process that occurs concurrently with root formation. The development of callus also shows that the cutting was healthy. The lack of rooting on the non-etiolated cuttings was not due to poor health, but some other unknown factor. We can conclude that callus development is significantly associated with etiolation.

Propagation via etiolated cuttings proved to be a simple and an effective method for bigtooth maple. The effects of etiolation on rooting percentage and number of roots per cutting in bigtooth maple have proven to be an indisputable asset in improving propagation in hard-to-root woody species. Further research is required on the other factors influencing leaf damage, leaf loss, and callus development among bigtooth maple cuttings. The impact of auxin levels and taking cuttings from non-juvenile sources are also in need of further experimentation. Although not all questions were answered in our experiment, the data on etiolation effects produced positive results that will be a significant contribution to improvements in the nursery and landscape industries in cloning ideal bigtooth maple specimens.

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CHAPTER V

SUMMARY AND CONCLUSIONS

The aesthetic, function, and fall color evaluations were valuable in selecting exceptional bigtooth maple specimens for clonal propagation. Free hand digital images taken from an airplane, synced with a GPS unit, and located using GeoSetter© and Google™ Earth was the most effective method in locating superior bigtooth maple trees. Other methods, such as personal communication and finding ideal trees in close proximity to an aerial-photographed tree, were shown to be advantageous in the selection process as well. The function and fall color evaluations aided immensely in filtering out the superior specimens from the inferior specimens. Six superior specimens with unique traits were selected. Five of the six selected specimen were cloned via budding. Future experiments will be carried out on these six ideal bigtooth maple specimens to prepare them for entering the landscape and nursery industries.

Clonal propagation needs to be improved in order for bigtooth maple to enter the nursery industry. Budding is a vital propagation method for introducing wild specimens into nursery environments and for carrying out further asexual propagation experiments. We performed various experiments to find the optimal time for budding bigtooth maple. A great deal of variation existed among our data, but a general trend was identified as the optimum time for grafting bigtooth maple buds. The July through mid-August months produced the highest bud take success and allows for greater improvement in the nursery industry.

Propagation via cuttings is another important type of clonal propagation that needs improvement among bigtooth maple. Etiolation was the main factor tested in our

cutting experiments. The relationship among etiolation and percentage of roots, number of roots per cutting, leaf damage, leaf loss, and callus development were all tested.

Etiolation was shown to have a significant effect on all of these elements. The etiolated cuttings showed an overall significantly greater percentage of roots, number of roots per cutting, leaf damage, leaf loss, and callus development than the non-etiolated cuttings.

The effects of etiolation on increased rooting show an undeniable advantage in improving propagation of bigtooth maple for the nursery industry.

Finding effective ways to select ideal bigtooth maple specimens, discovering the optimum time to bud bigtooth maple, and increasing root numbers on bigtooth maple cuttings are significant breakthroughs for the nursery and landscape industries. Bigtooth maple has tremendous economic potential and perfecting bigtooth maple propagation is a long-term effort. We expect these discoveries will improve the nursery and landscape industries.

APPENDICES

APPENDIX A.

Selection of bigtooth maple (*Acer grandidentatum* Nutt.)

APPENDIX A



Fig. A.1. Munsell color chart comparison with fall color of USU-ACGR-1022.

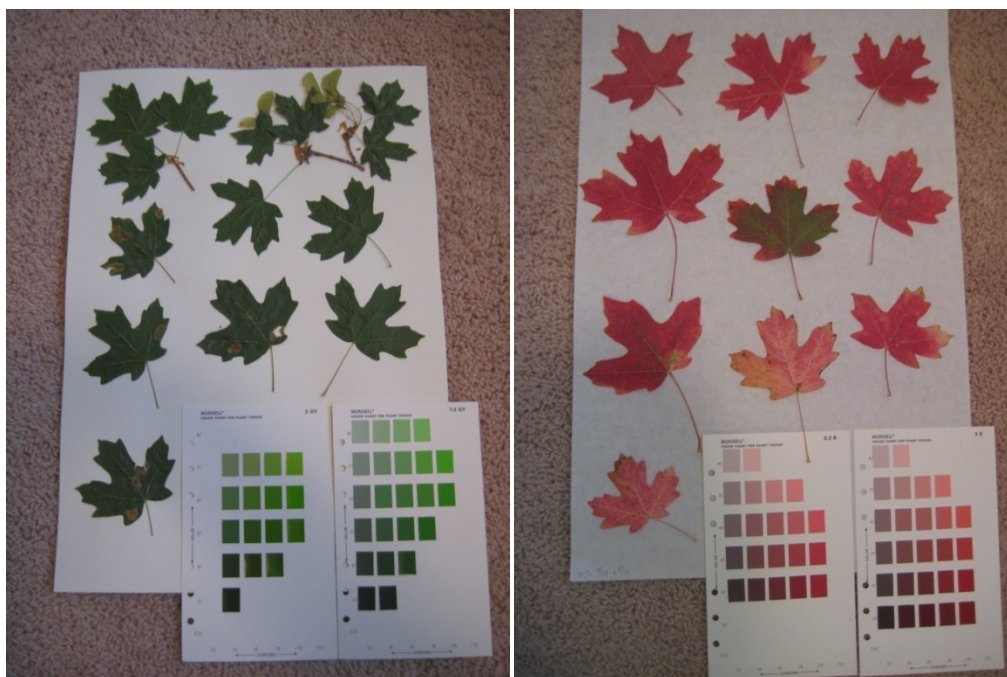


Fig. A.2 Munsell color chart comparison of summer and fall of USU-ACGR-1022.



Fig. A.3. Munsell color chart comparison with fall color of USU-ACGR-1032.

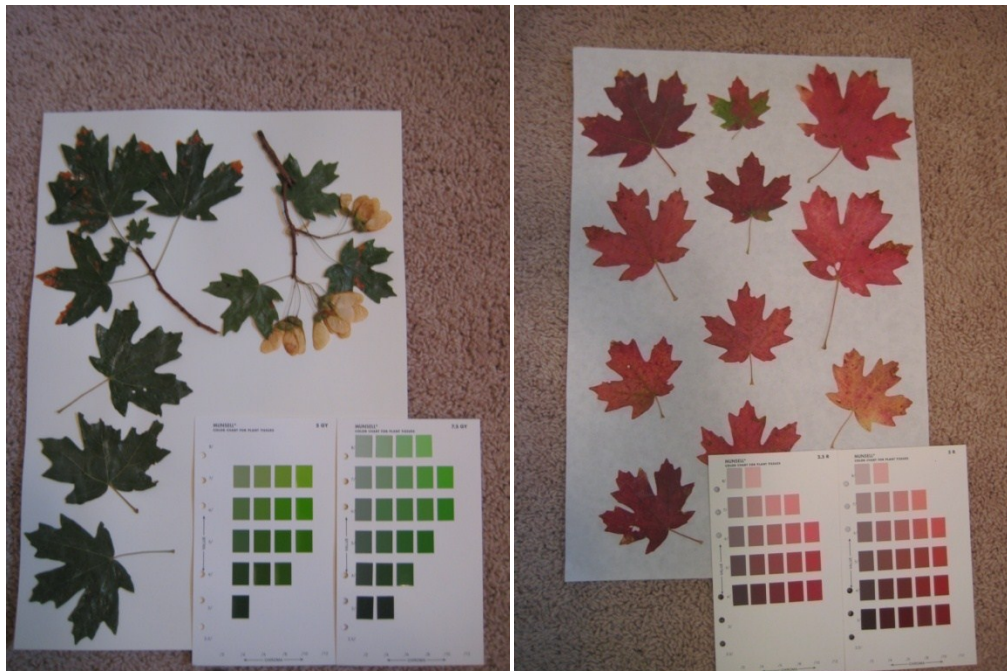


Fig. A.4. Munsell color chart comparison of summer and fall of USU-ACGR-1032.



Fig. A.5. Munsell color chart comparison with fall color of USU-ACGR-1034.

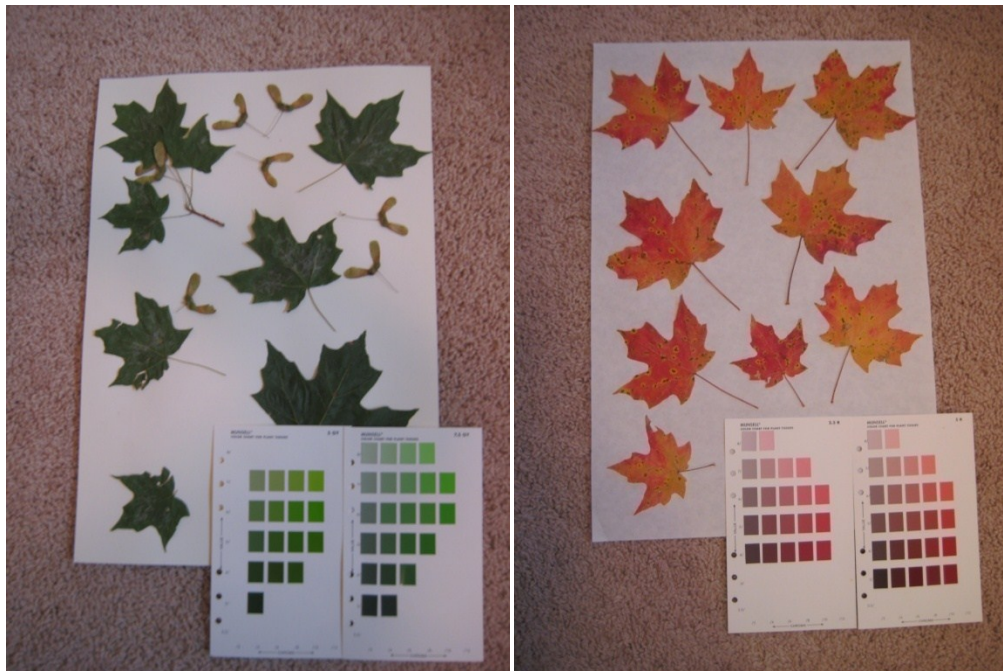


Fig. A.6 Munsell color chart comparison of summer and fall of USU-ACGR-1034.



Fig. A.7. Munsell color chart comparison with fall color of USU-ACGR-1036.

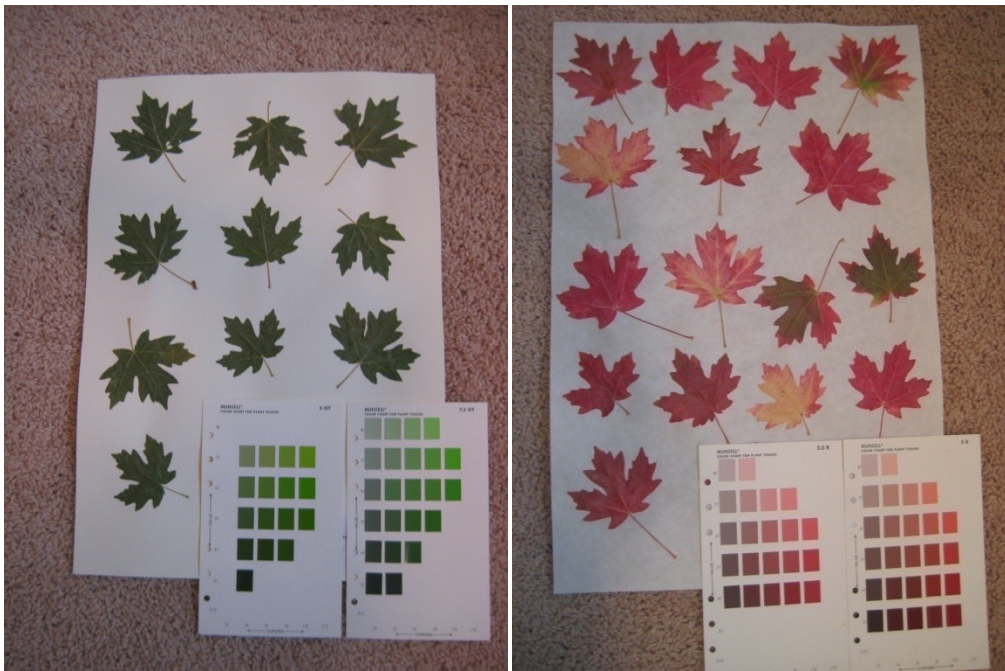


Fig. A.8 Munsell color chart comparison of summer and fall of USU-ACGR-1036.



Fig. A.9. Munsell color chart comparison with fall color of USU-ACGR-1038.

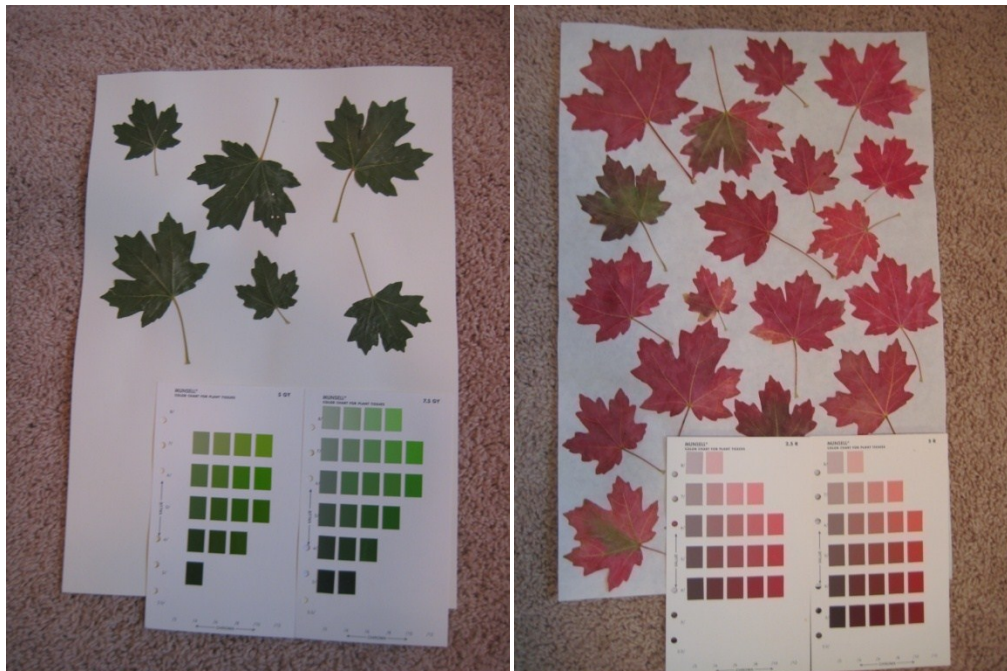


Fig. A.10 Munsell color chart comparison of summer and fall of USU-ACGR-1038.



Fig. A.11. Munsell color chart comparison with fall color of USU-ACGR-1041.

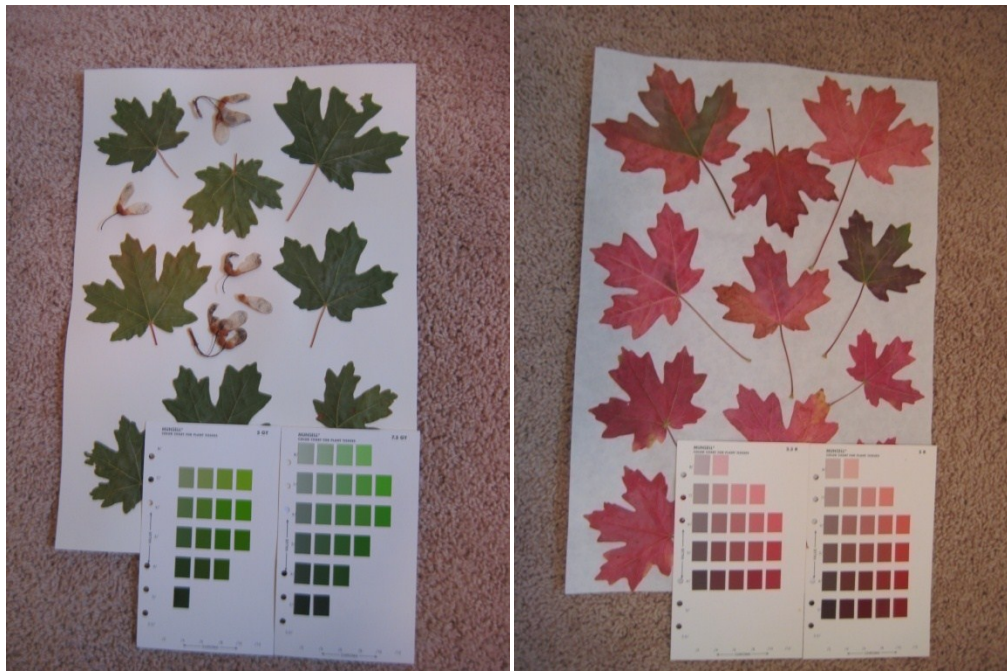


Fig. A.12 Munsell color chart comparison of summer and fall of USU-ACGR-1041.

APPENDIX B.

The effect of budding date on successful budding on bigtooth maple (*Acer grandidentatum* Nutt.)

APPENDIX B

Table B.1. Logistic regression statistical analyses on the effect of budding date on return budding success in 2006-07.

Logistic Regression of bud

Predictor Variables	Coefficient	Std Error	Coef/SE	P
Constant	1.61861	0.36750	4.40	0.0000
date2	-0.24548	0.05122	-4.79	0.0000
Deviance	217.42			
P-Value	0.0142			
Degrees of Freedom	174			

Convergence criterion of 0.01 met after 3 iterations

Cases Included 176 Missing Cases 4

Table B.2. Logistic regression statistical analyses on the effect of budding date on budding success with wild budwood in 2007-08.

Logistic Regression of bud

Predictor Variables	Coefficient	Std Error	Coef/SE	P
Constant	-2.35759	0.82579	-2.85	0.0043
date2	0.68722	0.24203	2.84	0.0045
Deviance	58.91			
P-Value	0.1344			
Degrees of Freedom	48			

Convergence criterion of 0.01 met after 3 iterations

Cases Included 50 Missing Cases 0

Table B.3. Logistic regression on statistical analyses on the effect of budding date on budding success with wild budwood in 2009-10.

Logistic Regression of Buds

Predictor				
Variables	Coefficient	Std Error	Coef/SE	P
Constant	-3.84324	1.11658	-3.44	0.0006
Date	1.86643	0.48186	3.87	0.0001
Deviance	39.05			
P-Value	0.9735			
Degrees of Freedom	58			

Convergence criterion of 0.01 met after 5 iterations

Cases Included 60 Missing Cases 0

Table B.4. Logistic regression statistical analyses on the effect of budding date on budding success with wild budwood on potted plants 2009-10.

Logistic Regression of bud

Predictor				
Variables	Coefficient	Std Error	Coef/SE	P
Constant	-3.43604	0.99453	-3.45	0.0006
days	0.05321	0.01997	2.66	0.0077
Deviance	55.78			
P-Value	0.5211			
Degrees of Freedom	57			

Convergence criterion of 0.01 met after 3 iterations

Cases Included 5 Missing Cases 0

APPENDIX C.

Effect of etiolation on rooting bigtooth maple (*Acer grandidentatum* Nutt.) cuttings

APPENDIX C

Table C.1. Two-sample t-test for the effect of etiolation on percentage of rooted cuttings with square root transformation in 2009.

Two-Sample T Tests for sqrt_EPcn vs sqrt_NPcn

Variable	N	Mean	SD	SE
sqrt_EPcn	10	0.9391	0.0589	0.0186
sqrt_NPcn	10	0.6700	0.1604	0.0507
Difference		0.2691	0.1208	0.0540

T-Tests for Mean Difference

Null Hypothesis: difference = 0

Alternative Hyp: difference <> 0

Method	Variances	DF	T	P	95% CI for Difference	
					Lower	Upper
Pooled	Equal	18	4.98	0.0001	0.1556	0.3826
Satterthwaite	Unequal	11.4	4.98	0.0004	0.1507	0.3876

Homogeneity of Variances	DF	F	P
Folded F Test	9,9	7.41	0.0032

Cases Included 20 Missing Cases 0

Table C.2. Two-sample t-test for the effect of etiolation on percentage of rooted cuttings with square root transformation in 2010.

Two-Sample T Tests for sqrtE vs sqrtN

Variable	N	Mean	SD	SE
sqrtE	16	0.9208	0.0873	0.0218
sqrtN	16	0.2422	0.2382	0.0596
Difference		0.6786	0.1794	0.0634

T-Tests for Mean Difference

Null Hypothesis: difference = 0

Alternative Hyp: difference <> 0

Method	Variances	DF	T	P	95% CI for Difference	
					Lower	Upper
Pooled	Equal	30	10.70	0.0000	0.5490	0.8081
Satterthwaite	Unequal	19.0	10.70	0.0000	0.5458	0.8113

Homogeneity of Variances	DF	F	P
Folded F Test	15,15	7.44	0.0002

Cases Included 32 Missing Cases 0

Table C.3. Percentage of rooted cuttings with etiolation and non-etiolation of bigtooth maple in 2009.

Tree	Etiolated	Etiolated %	Non-etiolated	Non-etiolated %
USU-ACGR-1001-1	11/12	92%	2/12	17%
USU-ACGR-1001-2	8/11	73%	2/10	20%
	19/23	83%	4/22	18%
USU-ACGR-1002-1	17/18	94%	7/19	37%
USU-ACGR-1002-2	10/13	77%	9/17	53%
USU-ACGR-1002-3	16/17	94%	8/15	53%
	43/48	90%	24/52	46%
USU-ACGR-1003-1	12/17	71%	6/20	30%
	12/17	71%	6/20	30%
USU-ACGR-1004-1	20/21	95%	12/24	50%
USU-ACGR-1004-2	17/19	89%	16/21	76%
	37/40	93%	28/45	62%
USU-ACGR-1005-1	15/15	100%	11/16	69%
USU-ACGR-1005-2	5/5	100%	2/3	67%
	20/20	100%	13/19	68%
OVERALL AVG	131/148	89%	75/158	47%

Table C.4. Percentage of rooted cuttings with etiolation and non-etiolation of bigtooth maple in 2010.

Rooting Percentages				
Tree #	Etiolation	Etiolation %	Non-etiolation	Non-Etiolation %
USU-ACGR-1001-1	9/10	90%	1/17	6%
USU-ACGR-1001-2	2/2	100%	0/7	0%
	11/12	92%	1/24	4%
USU-ACGR-1002-1	8/11	73%	0/12	0%
USU-ACGR-1002-2	9/12	75%	1/13	8%
USU-ACGR-1002-3	6/12	50%	2/12	17%
	23/35	66%	3/37	8%
USU-ACGR-1003-1	14/19	74%	4/19	21%
	14/19	74%	4/19	21%
USU-ACGR-1004-1	13/14	93%	8/20	40%
USU-ACGR-1004-2	25/26	96%	8/25	32%
	38/40	95%	16/45	36%
USU-ACGR-1005-1	23/24	96%	3/20	15%
	23/24	96%	3/20	15%
USU-ACGR-1009-1	10/10	100%	3/13	23%
USU-ACGR-1009-2	5/7	71%	0/7	0%
USU-ACGR-1009-3	5/6	83%	0/6	0%
USU-ACGR-1009-4	5/5	100%	0/5	0%
USU-ACGR-1009-5	2/2	100%	0/2	0%
USU-ACGR-1009-6	2/3	67%	0/3	0%
USU-ACGR-1009-7	4/4	100%	1/6	17%
	33/37	89%	4/42	10%
Overall:	142/167	85%	31/187	17%

Table C.5. ANOVA of effect of etiolation on number of roots 2009.

Analysis of Variance Table for logroots

Source	DF	SS	MS	F	P
Etiolated	1	30.6592	30.6592	187.22	0.0000
Error	294	48.1448	0.1638		
Total	295				

Note: SS are marginal (type III) sums of squares

Grand Mean 0.6518 CV 62.09

Table C.6. ANOVA of effect of etiolation on number of roots 2010.

Analysis of Variance Table for Rootno

Source	DF	SS	MS	F	P
Selection	6	177.98	29.66		
Etiolatio	1	3960.40	3960.40	241.05	0.0000
Error	346	5684.60	16.43		
Total	353				

Note: SS are marginal (type III) sums of squares

Grand Mean 3.6856 CV 109.98

Table C.7. Average number of roots per etiolated and non-etiolated cutting in 2009.

2009	Etiolated	Non-etiolated
ACGR-USU-1001	9.9	0.8
ACGR-USU-1002	10.6	2
ACGR-USU-1003	3.3	0.8
ACGR-USU-1004	15	3.6
ACGR-USU-1005	17.6	3.1

Table C.8. Average number of roots per etiolated and non-etiolated cutting in 2010.

Tree	Etiolated	Non-etiolated
USU-ACGR-1001	5.25	0.04
USU-ACGR-1002	4.17	0.41
USU-ACGR-1003	3.21	0.42
USU-ACGR-1004	11.05	1.07
USU-ACGR-1005	8.04	0.5
USU-ACGR-1009	8	0.33

Table C.9. Chi-square test for heterogeneity or independence of 2009 effect of etiolation on leaf damage.

Statistix 9.0
2:49:30 PM

compiled data, 7/13/2010,

**Chi-Square Test for Heterogeneity or Independence
for 1 = Etiolated Leafdamag**

Etiolated		Leafdamag		N	Y			
		N	Y					
N	Observed	104	52	156	67%	33%		
	Expected	58.69	97.31					
	Cell Chi-Sq	34.97	21.10					
Y	Observed	10	137	147	7%	93%		
	Expected	55.31	91.69					
	Cell Chi-Sq	37.12	22.39					
		114	189	303				
Overall Chi-Square		115.57						
P-value		0.0000						
Degrees of Freedom		1						

Table C.10. Chi-square test for heterogeneity or independence of 2010 effect of etiolation on leaf damage.

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COMPILED 2010, 7/13/2010,

**Chi-Square Test for Heterogeneity or Independence
for 1 = Etiolatio LfDam**

Etiolatio		LfDam		N	Y			
		N	Y					
N	Observed	124	63	187	66%	34%		
	Expected	74.48	112.52					
	Cell Chi-Sq	32.92	21.79					
Y	Observed	17	150	167	10%	90%		
	Expected	66.52	100.48					
	Cell Chi-Sq	36.86	24.40					
		141	213	354				
Overall Chi-Square		115.97						
P-value		0.0000						
Degrees of Freedom		1						

Table C.11. ANOVA of 2009 effect of etiolation on leaf loss.

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compiled data, 7/13/2010,

Analysis of Variance Table for LfDiff

Source	DF	SS	MS	F	P
Etiolated	1	33.114	33.1141	45.73	0.0000
Variety	4	42.426	10.6065	14.65	0.0000
Error	289	209.253	0.7241		
Total	294				

Note: SS are marginal (type III) sums of squares

Grand Mean 1.0207 CV 83.37

Table C.12. ANOVA of 2010 effect of etiolation on leaf loss.

Statistix 9.0
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COMPILED 2010, 7/13/2010,

Analysis of Variance Table for LfDiff

Source	DF	SS	MS	F	P
Etiolatio	1	66.046	66.0457	86.50	0.0000
Tree	5	80.854	16.1708	21.18	0.0000
Error	347	264.936	0.7635		
Total	353				

Note: SS are marginal (type III) sums of squares

Grand Mean 1.5035 CV 58.12

Table C.13. Chi-square test for heterogeneity or independence of 2009 effect of etiolation on callus.

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compiled data, 7/13/2010,

**Chi-Square Test for Heterogeneity or Independence
for 1 = Etiolated Callus**

Etiolated		Callus				
		0	1			
N	Observed	10	145	155	6%	94%
	Expected	5.24	149.76			
	Cell Chi-Sq	4.33	0.15			
Y	Observed	0	141	141	0%	100%
	Expected	4.76	136.24			
	Cell Chi-Sq	4.76	0.17			
		10	286	296		

Overall Chi-Square 9.41
P-value 0.0022
Degrees of Freedom 1

CAUTION: 1 cell(s) have expected values less than 5.0

Table C.14. Chi-square test for heterogeneity of independence of 2010 effect of etiolation on callus.

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COMPILED 2010, 7/13/2010,

**Chi-Square Test for Heterogeneity or Independence
for 1 = Etiolatio Callus**

Etiolatio		Callus				
		N	Y			
N	Observed	46	140	186	25%	75%
	Expected	26.42	159.58			
	Cell Chi-Sq	14.51	2.40			
Y	Observed	4	162	166	2%	98%
	Expected	23.58	142.42			
	Cell Chi-Sq	16.26	2.69			
		50	302	352		

Overall Chi-Square 35.86
P-value 0.0000
Degrees of Freedom 1